Title: A spectrophotometric investigation into the catechins in *Camellia sinensis*.

Research Question: How does the concentration of catechins in *Camellia sinensis* (9.9, 10.9, 12.1, 13.6, 15.6μM) affect the change in absorbance when shielding ascorbic acid from oxidization as measured by spectrophotometry?

IB subject: Chemistry

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1 Introduction

Camellia sinensis, a shrub in the flowering plant family *Theaceae*, is traditionally used to produce caffeinated teas such as black and green tea (Washington State University). According to the Chinese legend, the discovery of tea in China dates to around 2700 BCE (Britannica). Since the 13th century, where tea cultivation began to spread to other parts of the world, green tea has become an important commodity and is engraved in the cultures of many countries such as Japan, China, Britain, and Indonesia (Universitas Airlangga). As more discoveries point out the health benefits and antioxidant properties of green tea such as preventing cellular damage, lowering risk of heart and liver disease, regulating blood sugar levels, promoting joint health, and supporting weight loss, the product has become widely used and marketed in dietary supplements and skin care.

2 Research question

Studies in health, pharmaceutical, and cosmetic industries often use green tea to understand how antioxidant efficacy in certain foods affect its nutritional value or how to develop certain creams or supplements with optimized anti-aging properties (Prasanth et al., 2019).

This led to me formulate the research question for this study:

"How does the concentration of catechins in *Camellia sinensis* (9.9, 10.9, 12.1, 13.6, 15.6µM) affect the change in absorbance when shielding ascorbic acid from oxidization as measured by spectrophotometry?"

Through varying catechin concentrations and monitoring absorbance changes, this experiment seeks to show how the concentration of catechins in a green tea solution influences the oxidation rate. UV-Visible spectrophotometry employs an empirical role in understanding the catechin's ability to shield ascorbic acid from oxidation by hydrogen peroxide, hence determining its antioxidant properties.

3 Background

3.1 Antioxidants in green tea

In East Asia, green tea is renowned for its health benefits, particularly due to its rich content of antioxidants. Antioxidants play a crucial role in mitigating oxidative stress, a condition characterized by an imbalance between free radicals and antioxidants in the body. Green tea is concentrated with polyphenols, such as flavonoids, theaflavins, and catechins (Musial, 2020). Specifically, Catechins are antioxidants and secondary metabolites synthesized by *Camellia sinensis* as a defense mechanism against environmental stressors such as UV radiation and pathogens (Truong et al., 2022). The primary catechins found in green tea include Epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC), and epicatechin (EC). Since green tea leaves go through a lot less processing such as pan-firing and steaming, it has a higher content of EGCG (as displayed in Figure 1) compared to black tea (Abiri et al., 2023). Heat application denatures the enzyme polyphenol oxidase, which stops oxidation and preserves catechins such as EGCG in their original form (Ke et al., 2020). Therefore, with abundance in EGCG, green tea is more capable of neutralizing free radicals as EGCG has great numbers of hydroxyl groups in its structure (Munoz-Munoz J.L. et al., 2008). The compound functions as an antioxidant by donating hydrogen atoms or electrons to reactive

oxygen species (ROS) and free radicals which can cause oxidative stress (Rong, 2012). Oxidative stress is the imbalance between reactive oxygen species (ROS) and reactive nitrogen species (RNS) that can create damage to the DNA and oxidation proteins inside cells. When ROS is generated, the resulting oxidative stress can disrupt the function of normal mechanisms and cellular signaling (He et al., 2018). EGCG can minimize, prevent, or delay the harm that is created. The compound can also block certain pathways that trigger chronic inflammation and activate enzymes to naturally detoxify harmful substances.

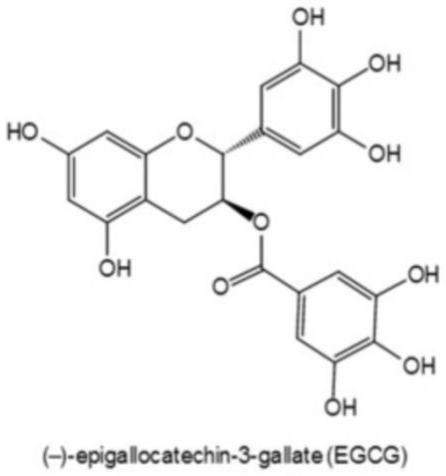


Figure 1: Molecular model of EGCG. *Animal Nutrition.*

https://www.sciencedirect.com/science/article/pii/S2405654520300032

3.2 Hydrogen peroxide

The antioxidant activity of catechins can be quantified using spectrophotometric methods, particularly by measuring changes in absorbance when they interact with oxidizing agents such as hydrogen peroxide (H_2O_2). When catechins compete with other antioxidants—such as ascorbic acid (vitamin C)—for oxidation by hydrogen peroxide H_2O_2, the reaction kinetics can

provide insight into their relative antioxidant capacities. The reactive oxygen species hydrogen peroxide's incredibly weak and unstable oxygen-oxygen single bond, as shown in Figure 2, will easily break and reduce into water as seen in Equation 1.

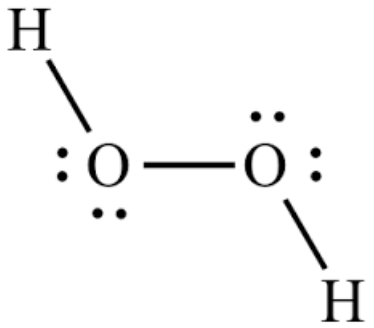


Figure 2: Molecular model of hydrogen peroxide. *UCLA – Chemistry and Biochemistry.*

https://www.chem.ucla.edu/~harding/IGOC/H/hydrogen_peroxide.html

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$$

Equation 1: Reduction reaction equation of hydrogen peroxide. *JSM Learn.*

https://www.youtube.com/watch?v=sIbIMclXeKI

The bond breaking also releases highly reactive free radicals (ReAgent). As one of the major types of ROS, hydrogen peroxide regulates cell apoptosis, autophagy, and can even damage the central nervous system. Although ROS is essential for the intracellular signaling in the central nervous system, excessive ROS accumulation can result in cellular oxidative damage (He et al., 2018).

3.3 Ascorbic acid

Ascorbic acid (Figure 3), more commonly known as vitamin C, is a well-known water-soluble antioxidant that protects the oxidation of macromolecules inside the body such as proteins, fats, and DNA. It is naturally present in fresh fruits and vegetables. Like catechins, it donates electrons to neutralize free radicals, forming dehydroascorbic acid ($C_6H_6O_6$) as an oxidation product (Raman, 2023) as shown in Equation 2.

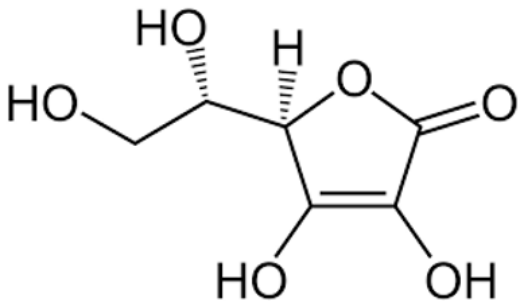


Figure 3: Molecular model of ascorbic acid. *American Chemical Society. https://www.acs.org/molecule-of-the-week/archive/a/ascorbic-acid.html*

$$C_6H_8O_6 \rightarrow C_6H_6O_6 + 2H^+ + 2e^-$$

Equation 2: Oxidation reaction equation of ascorbic acid. *Research Gate. https://www.researchgate.net/figure/Chemical-structure-of-ascorbic-acid-and-dehydroascorbic-acid-as-depicted-through-a-redox_fig1_312229647*

3.4 Spectrophotometry

Spectrophotometry is a widely used technique in chemistry that measures how much a chemical substance absorbs light by measuring the intensity of light that passes through the solution, hence determining the number of photons absorbed (Chemistry LibreTexts). It can assess antioxidant

activity by tracking changes in absorbance at specific wavelengths. The result will be a quantitative value of the absorbance between 0 and 1 absorbance units (AU). For this experiment, in the presence of hydrogen peroxide, catechins and ascorbic acid undergo oxidation, leading to a decrease in the concentration of the reducing agents and a corresponding decrease in absorbance (Chen et al., 2020). Specifically, a UV-Visible spectrophotometer will be used as it is able to measure wavelengths in the ultraviolet range (185 - 400 nm) and the visible range (400 - 700 nm) of the electromagnetic radiation spectrum. This is because catechins, which are phenols, are oxidized into quinones (see Figure 4). Through using the lambda max (λ_{max}) published by Munoz-Munoz J.L. et al. on the Journal of Agricultural and Food Chemistry and by Mohammed et al. on the Journal of Kirkuk University, the wavelength which quinones have the strongest photon absorption is shown to be at around 280nm. Hence, the wavelength used for this experiment is controlled at 288nm.

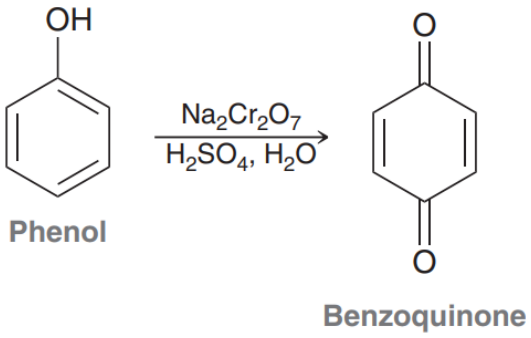


Figure 4: Oxidation of phenol to benzoquinone. *Chemistry Stack Exchange.*

https://chemistry.stackexchange.com/questions/119700/what-is-the-mechanism-of-oxidation-of-phenol-to-benzoquinone

3.5 Beer Lambert law

The Beer-Lambert law, a fundamental principle in spectroscopy widely used in analytical chemistry, establishes a correlation between absorbance of light (absorbance value from the spectrophotometer) and concentration of catechins in the solution. It states that a given material sample path length and concentration of the sample are directly proportional to the absorbance of light by a solution (Edinburgh Instruments).

$$A = \varepsilon c l$$

Equation 3: Equation for Beer-Lambert law. *Edinburgh Instruments*.

https://www.edinst.com/resource/the-beer-lambert-law/

As shown in Equation 3, A is the amount of light absorbed for a particular wavelength by the sample, ε is the molar absorption coefficient ($L \cdot mol^{-1} \cdot cm^{-1}$), c is the molar concentration of the solution (mol/L), and l is the optical path length (cm) which is the width of the cuvette. Since standard spectrophotometers use cuvettes with a width of 1cm, l is always assumed to be 1. In this equation, as A increases and approaches 1, more light is being absorbed by the sample and less is being transmitted (Chemistry LibreTexts).

4 Methodology

4.1 Chemical equation

The experiment will carry out through the following balanced reaction equation:

$$C_6H_8O_6 + 2H_2O_2 + C_{15}H_{14}O_6 \rightarrow C_6H_6O_6 + 4H_2O + C_{15}H_{12}O_6$$

Equation 4: Oxidization of catechin and ascorbic acid using hydrogen peroxide

In this reaction, ascorbic acid ($C_6H_8O_6$) gets oxidized to dehydroascorbic acid ($C_6H_6O_6$). Catechins ($C_{15}H_{14}O_6$), due to their highly reactive -OH groups, are easily able to donate electrons to oxidizing agents like hydrogen peroxide, allowing catechins to oxidize into quinones. Finally, hydrogen peroxide (H_2O_2) gets reduced to water (H_2O) by ascorbic acid and catechins (National Institutes of Health).

4.2 Controlled variables

Table 1: Controlled variables of the study

Variable	Value	Justification
Concentration of ascorbic acid solution (mol/L)	1.325×10^{-2} $\pm 0.02 \times 10^{-2}$	As the independent variable is the concentration of catechins, the other reagents should be controlled to measure the dependent variable accurately. The amount is measured with an electric scale for each experiment.

Concentration of hydrogen peroxide (%)	6 ± 0.1	The concentration of hydrogen peroxide will affect the extent of oxidation. The concentration of reactants other than catechins needs to be kept consistent to isolate the independent variable.
Wavelength of spectrophotometer (nm)	288 ± 0.5	Data is collected in the ultraviolet range as the activity of EGCG is strongest in the UV range and the antioxidant activity of quinones is detected at a wavelength under 400nm (World Health Organization). The selected wavelength is based off the absorption maxima literature value published by Munoz-Munoz J.L. et al. on the Journal of Agricultural and Food Chemistry and by Mohammed et al. on the Journal of Kirkuk University. The wavelength stays consistent for all samples to have accurate absorbance values. Additionally, a quartz cuvette is used to effectively and precisely transmit UV light.
Time taken for each trial (minutes)	5 ± 0.1	Ascorbic acid and catechin will continue reducing in the presence of the oxidizing

		agent without a time limit. Results need to be recorded over a specific and limited time period that is consistent across all samples to accurately measure the rate. A stopwatch is used to monitor the duration taken for each sample.
Temperature (°C)	24 ± 1	Controlling the temperature of the environment minimizes changes in reaction kinetics to reduce irregularities as an increase in temperature makes the reaction proceed more rapidly according to the collision theory (Chemistry LibreTexts). All experiments are conducted in a temperature-controlled lab environment. Reagents are all stored at room temperature away from direct sunlight. Hydrogen peroxide is stored at around 5°C.

4.3 Preparation of catechin solutions

Table 2: Catechin solution concentrations

Catechin concentration (±0.1μM)	Mass of Camellia sinensis (green tea) powder (±0.005g)	Volume of distilled water (±0.01L)
15.6	0.05	0.7
13.6	0.05	0.8
12.1	0.05	0.9
10.9	0.05	1
9.9	0.05	1.1

Using EGCG's estimated molecular weight of 458.4 g/mol (Yang et al., 2017) and an approximation of green tea powder containing 10% catechin (Nain et al., 2022), an example calculation of the solution concentrations is shown below.

$$0.05g\ (Mass\ of\ powder) * 0.10(Catechin\ percentage)$$

$$= 0.005g\ (Mass\ of\ catechins\ in\ 0.05g\ green\ tea\ powder)$$

$$\frac{0.005g}{458\ g/mol\ (Molecular\ weight)} = 1.092 * 10^{-5}\ mol\ (Moles\ of\ catechins)$$

$$\frac{1.092 * 10^{-5}\ mol}{0.7L\ (Volume\ of\ solution)} = 15.6\ \mu M\ (\text{Molar concentration})$$

4.4 Procedure

1. Set up the spectrophotometer: Open the Logger Pro app on a laptop and plug in the UV-Visible spectrophotometer. Once the spectrophotometer is warmed up, fill a cuvette with distilled water and insert it into the spectrophotometer for a controlled trial. Since water

has little to no absorbance, the controlled trial helps eliminate any interference and ensures the spectrophotometer is calibrated and only measures the absorbance of the sample of interest.

2. Prepare the ascorbic acid solution: Measure and dissolve 0.35g of ascorbic acid in 150ml of water.
3. Prepare the green tea solutions: Measure and dissolve the Camellia sinensis (green tea) powder according to Table 2 in five separate large beakers.
4. In a small separate beaker, measure 5g of the ascorbic acid solution and 5g of one of the green tea solutions. Mix until combined.
5. Add 1g of 6% hydrogen peroxide. Swirl and mix the solution, then transfer it into a quartz cuvette.
6. Insert the cuvette into the spectrophotometer and record its initial absorbance value at 288nm. Start a stopwatch.
7. Record the changing absorbance value of the solution in one-minute intervals. Record for a total of 5 minutes.
8. Dispose of the solution in a waste beaker and rinse the cuvette. Then, repeat steps 4-8 four more times with the same green tea solution concentration.
9. Repeat steps 4-9 with the other green tea solutions diluted to different concentrations.

4.5 Safety considerations

Hydrogen peroxide is corrosive to skin, eyes, and mucous membranes at concentrations higher than 10% (ATSDR, 2014). This experiment uses 6% hydrogen peroxide, which can

still cause irritation. Hence, plastic gloves and lab goggles need to be worn during the experiment when handling hydrogen peroxide.

5 Qualitative and quantitative data

Table 3: Qualitative observations

	Ascorbic acid solution	Camellia sinensis solution	Hydrogen peroxide	Final combined solutions after 5 minutes
Qualitative observations	Colorless liquid.	Light green liquid.	Colorless liquid with small air bubbles and faint odor.	Light green liquid with some dark green precipitate at the bottom of the cuvette. More precipitate observed for solutions with higher green tea concentration.

Table 4: Raw data for change in absorbance over time when catechin concentration is 15.6μM

(Only one raw data table is included below as reference. The remaining raw data tables are displayed in Appendix.)

		Time						
		0 min	1 min	2 min	3 min	4 min	5 min	Rate of change (AU/min)
Absorbance values (±0.001AU)	Trial 1	0.474	0.343	0.248	0.161	0.106	0.051	-0.0832
	Trial 2	0.365	0.301	0.228	0.164	0.107	0.039	-0.0650
	Trial 3	0.449	0.347	0.293	0.188	0.118	0.063	-0.0778
	Trial 4	0.376	0.306	0.203	0.113	0.038	0.004	-0.0787
	Trial 5	0.413	0.316	0.210	0.140	0.068	0.032	-0.0777

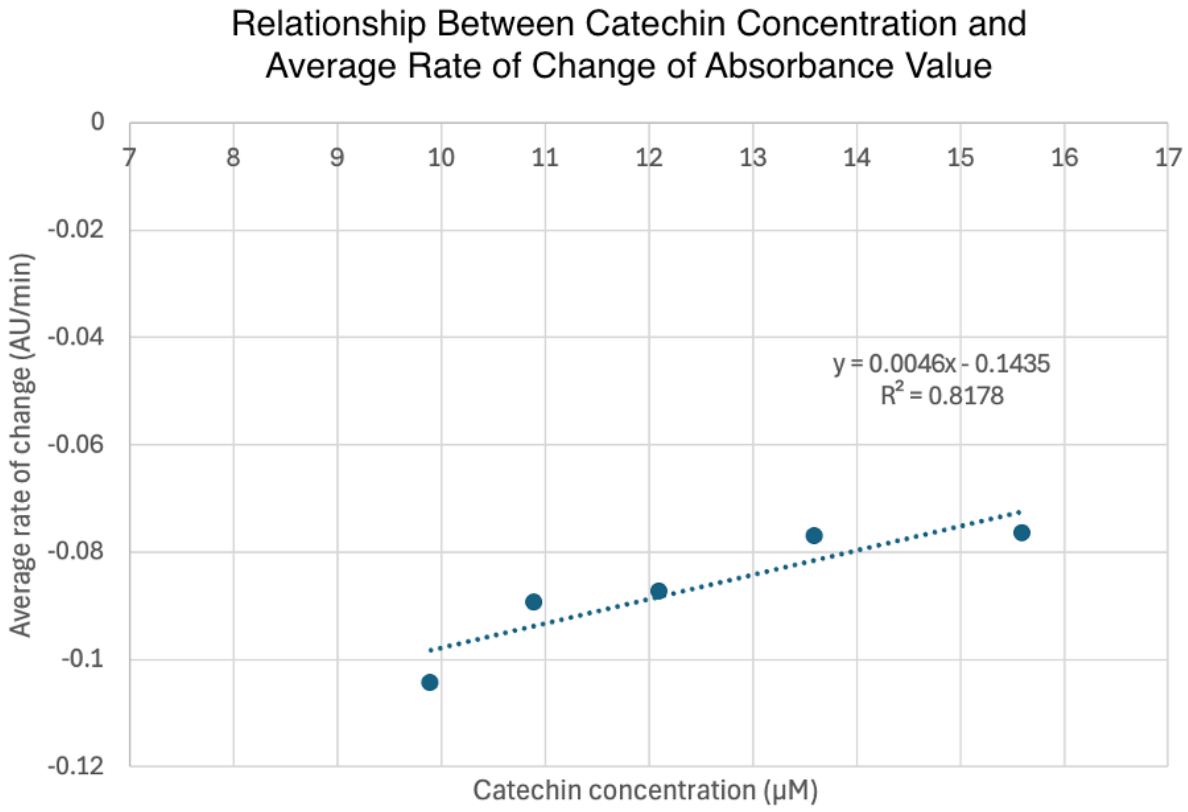
Method of finding rate of change:

The rate of change is calculated by plotting each data point on a graph and then generating a trendline. The slope of the trendline is set as the rate of change and recorded down.

Table 5: Effect of catechin concentration on average rate of change of absorbance

Catechin concentration (±0.1μM)	Average rate of change (±0.001AU/min)
15.6	-0.0765
13.6	-0.0770
12.1	-0.0873
10.9	-0.0893
9.9	-0.1043

Figure 5: Scatter plot of relationship between catechin concentration and average rate of change of absorbance.



6 Data Analysis

6.1 Analysis of the graph

Through the Figure 5 scatter plot graph with the independent variable catechin concentration as the x-axis, and the dependent variable average rate of change of absorbance as the y-axis, it is apparent that there is a relatively strong positive correlation between the concentration and the average change in absorbance as the five data points have a Pearson's correlation coefficient (calculated by square rooting the R^2 value on Excel) of 0.8178. The graph also has a linear trendline with a slope of 0.0046, showing that there is a positive upward trend. This means that as catechin concentration increases, the average rate of change of absorbance decreases. In other words, the graph of the rate of change obtains a flatter slope, hence absorbance values are decreasing at a slower rate. Moreover, Figure 5 does not display any significant outliers, showing the reliability and precision of the data.

6.2 Uncertainty and error calculations

Using Table 4, the average rate of change for when the concentration is 15.6μM is calculated to be -0.0765 ±0.001AU/min. The absolute uncertainty is 0.001AU/min. Hence, the relative uncertainty is calculated to be 1.31% with Equation 5.

$$\frac{Absolute\ uncertainty}{Measured\ value} * 100\% = Relative\ Uncertainty$$

Equation 5: Formula for relative uncertainty. *ThoughtCo.*

https://www.thoughtco.com/definition-of-relative-uncertainty-605611

The literature value I have chosen has a molar absorptivity of 0.0323 L $mol^{-1}cm^{-1}$ when measured at a wavelength of 280nm (Mohammed et al., 2009). Now, I will calculate the molar absorption coefficient for this experiment with the Beer-Lamber law (Equation 3) and the first column of data points when the concentration is 15.6μM.

$$A = \varepsilon * c * l$$

$$\frac{0.474 + 0.343 + 0.449 + 0.376 + 0.413}{6} = 0.411AU \text{ (average)}$$

$$0.411 = \varepsilon * 15.6 * 1$$

$$\varepsilon = 0.0263\ L\ mol^{-1}cm^{-1}$$

$$\frac{|0.0323 - 0.0263|}{0.0323} * 100\% = 18.6\%$$

Therefore, the percentage error, calculated by finding the absolute value of the difference between the theoretical value and the experimental value, then dividing the difference by the theoretical value (Vedantu), is 18.6%. The smaller the percentage error, the higher the accuracy of the data. A percentage error below 5% is considered very accurate and close to the true value (Vendantu). Hence, the percentage error for these specific trials shows that the data is still slightly questionable and not completely accurate and reliable.

7 Conclusion

This study quantitatively examined how varying concentrations of catechins from *Camellia sinensis* affect the oxidation of ascorbic acid in the presence of hydrogen peroxide, using

spectrophotometric analysis to measure changes in absorbance. As the concentration of catechin increased, the rate of the change in absorbance decreased, meaning the ascorbic acid oxidized slower. Hence, there is an inverse relationship between catechin concentration and ascorbic acid oxidation rate.

The observed reaction dynamics can be explained through fundamental principles of chemical kinetics and redox chemistry. Catechins, particularly EGCG, possess multiple phenolic hydroxyl groups that readily donate electrons to oxidizing agents like hydrogen peroxide. When present in sufficient concentrations, these polyphenolic compounds effectively compete with ascorbic acid for available oxidants, thereby protecting the ascorbic acid from oxidation. This protective effect diminishes as catechin concentration decreases, allowing more hydrogen peroxide to react with ascorbic acid instead. The spectrophotometric measurements at 288 nm effectively captured the changes in absorbance.

Hence, this experiment shows that *Camellia sinensis* has strong antioxidant properties as it can delay the oxidation of ascorbic acid, a compound which can very easily oxidize in light, high temperatures, heavy metals, alkali, and more.

8 Evaluation

8.1 Limitations
- The absorbance value (in absorbance units) is a quantitative method that quantifies the strength of the antioxidant. This is a limitation as the spectrophotometer can only

measure the amount of light passing through the sample but cannot measure the exact effectiveness of the antioxidant.

- The final graph is an average of the different values of rate of change for the same concentration. This is a limitation as calculating the average may erase outliers, leading to an inaccurate display of the data.

8.2 Weaknesses

Systematic errors	Random errors
- The green tea powder used may contain other contaminants and ingredients other than pure catechin powder. This could have affected the chemical reaction and altered the results, leading to inaccuracy. Pure catechin can be used instead of green tea powder. - To calculate the molar concentration of catechins, an estimation of the percentage of catechins in green tea is used (10%). Yet, the exact concentration varies depending on how the green tea is processed. Hence, using pure catechin powder can prevent inaccuracy, so the exact concentration used for the experiment is known.	- Analog instruments such as a volumetric flask were used to measure volume. This could have resulted in random error due to inaccuracy of the human eye. Digital instruments are preferred over analog instruments to decrease uncertainty and error.

- The quartz cuvette used to determine absorbance can easily be scratched. Fingerprints can also affect the transparency of the cuvette. Hence, always avoid touching the sides that light passes through and carefully examine the cuvette for scratches before the experiment. - Starting the oxidation by mixing the hydrogen peroxide into the ascorbic acid and green tea solution, pouring the solution into the cuvette, starting the timer, and starting data collection with the spectrophotometer did not occur at the same moment, hence the delay could have caused a lack of accuracy in the data. To collect more accurate data, data collection needs to start at the exact same moment the hydrogen peroxide comes into contact with the ascorbic acid and green tea.	- Only five variations of the independent variable were tested and graphed. Repeating trials or testing other different concentrations (more concentrated and less concentrated catechin solutions) can increase precision and show a clearer, more significant trend.

8.3 Comparison between systematic errors and random errors

Overall, there are more systematic errors than random errors within this experiment. This suggests that many fundamental issues may have occurred during the experimental set up and methodology. It indicates that any inaccuracies would have occurred not from unpredictable fluctuations, but from a consistent, reproducible bias the skews the final data away from the true

value. Therefore, the flawed experimental design should be edited according to the identified systematic errors to reduce and eliminate the possibility of unreliable results.

9 Appendix

Table 6: Change in absorbance over time when catechin concentration is 13.6μM

		Time						
		0 min	1 min	2 min	3 min	4 min	5 min	Rate of change (AU/min)
Absorbance values (± 0.001AU)	Trial 1	0.482	0.387	0.269	0.187	0.117	0.065	-0.0851
	Trial 2	0.453	0.347	0.259	0.182	0.116	0.057	-0.0786
	Trial 3	0.392	0.292	0.199	0.125	0.061	0.022	-0.0748
	Trial 4	0.443	0.344	0.233	0.146	0.103	0.048	-0.0796
	Trial 5	0.483	0.367	0.336	0.246	0.184	0.143	-0.0668

Average rate of change: -0.0770 AU/min

Table 7: Change in absorbance over time when catechin concentration is 12.1µM

		Time						
		0 min	1 min	2 min	3 min	4 min	5 min	Rate of change (AU/min)
Absorbance values (± 0.001AU)	Trial 1	0.619	0.505	0.401	0.305	0.223	0.153	-0.0935
	Trial 2	0.517	0.340	0.233	0.144	0.095	0.038	-0.0920
	Trial 3	0.429	0.284	0.189	0.105	0.056	0.017	-0.0808
	Trial 4	0.535	0.408	0.296	0.211	0.146	0.102	-0.0867
	Trial 5	0.447	0.327	0.217	0.126	0.083	0.026	-0.0837

Average rate of change: -0.0873 AU/min

Table 8: Change in absorbance over time when catechin concentration is 10.9µM

		Time						
		0 min	1 min	2 min	3 min	4 min	5 min	Rate of change (AU/min)
Absorbance values (± 0.001AU)	Trial 1	0.577	0.433	0.311	0.239	0.165	0.139	-0.0876
	Trial 2	0.534	0.353	0.268	0.180	0.132	0.088	-0.0852
	Trial 3	0.637	0.499	0.366	0.260	0.179	0.137	-0.1019
	Trial 4	0.437	0.323	0.196	0.130	0.063	0.032	-0.0820
	Trial 5	0.644	0.531	0.391	0.310	0.246	0.205	-0.0895

Average rate of change: -0.0893 AU/min

Table 9: Change in absorbance over time when catechin concentration is 9.9µM

		Time						
		0 min	1 min	2 min	3 min	4 min	5 min	Rate of change (AU/min)
Absorbance values (± 0.001AU)	Trial 1	0.683	0.433	0.314	0.205	0.142	0.109	-0.1148
	Trial 2	0.534	0.353	0.268	0.180	0.132	0.110	-0.8480
	Trial 3	0.598	0.478	0.354	0.201	0.139	0.074	-0.1048
	Trial 4	0.547	0.439	0.299	0.189	0.073	0.029	-0.1036
	Trial 5	0.668	0.572	0.491	0.370	0.266	0.101	-0.1134

Average rate of change: -0.1043 AU/min

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