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Comparative antioxidant analysis using the iodine clock reaction: A kinetic approach

Hassan Darwish [1, *], Danielle Parker [1], Evan Brewer [1], Harajit Rao [1], Julisa Laris [1], Hiba Al Lawati [2], Hagar M. Mohamed [3, 3], Manohar Noone [4] and Una Gibbons [5]

[1] *Physical Sciences Program, School of Arts, Sciences, and Education, Ivy Tech Community College, South Bend, Indiana, USA.*
[2] *School of Biomedical Sciences, Faculty of Health, Queensland University of Technology, Australia.*
[3] *Department of Applied Medical Chemistry, Medical Research Institute, Alexandria University, Alexandria, Egypt*
[3] *Department of Medical Laboratory Analysis, Faculty of Medical & Health Sciences, Liwa College, Abu Dhabi, UAE*
[4] *Noon's Clinic, Muscat, Oman.*
[5] *Royal Hospital, Oman (Former), Kilkenny, Ireland.*

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Abstract

Aim: This study aims to evaluate the antioxidant capacity of black tea, turmeric, melatonin, and chamomile/lavender tea using the iodine clock reaction, a kinetic method for assessing antioxidant activity.

Background: Oxidative stress, caused by an imbalance between free radicals and antioxidants, contributes to chronic diseases, including cardiovascular diseases and neurodegenerative disorders. Antioxidants play a crucial role in mitigating oxidative damage.

Methods: The iodine clock reaction was utilized to assess antioxidant activity by measuring the delay in the oxidation process. Reaction times were recorded for each antioxidant sample, and statistical analysis (P-test) was performed to determine significance compared to the control.

Results: Black tea exhibited the longest reaction delay (612.47s), indicating the highest antioxidant potential, followed by melatonin (344.75s), chamomile/lavender tea (248.98s), and turmeric (200.56s). Statistical analysis confirmed significant differences in antioxidant efficiencies ($P < 0.05$).

Conclusion: The results suggest that black tea is the most potent antioxidant among the tested samples, supporting its health benefits. The iodine clock reaction proves to be a reliable method for antioxidant evaluation. Further research should explore additional antioxidants and optimize experimental conditions.

Keywords: Antioxidant activity; Iodine clock reaction; Oxidative stress; Chemical kinetics; Dietary antioxidants; P-test analysis; Free radicals

1. Introduction

Oxidative stress results from an excess of reactive oxygen species (ROS), leading to cellular damage and disease progression, including cancer, cardiovascular diseases, and neurodegenerative disorders (Halliwell & Gutteridge, 2015; Valko et al., 2007). Antioxidants neutralize free radicals, preventing oxidative damage. While numerous antioxidants exist, their effectiveness varies based on molecular structure and reaction conditions (Prior et al., 2005; Apak et al., 2007).

* Corresponding author: Hassan Darwish

The iodine clock reaction, a well-established chemical kinetics experiment, provides a means to assess antioxidant potential by measuring the time delay before the appearance of a blue-black starch-iodine complex (Landolt, 1886; Gao et al., 2019). While previous studies have utilized the iodine clock reaction for educational demonstrations, this study applies the method to evaluate antioxidant activity, making it a novel approach in the field of antioxidant research

2. Material and methods

2.1. Chemicals and Reagents

- Hydrogen peroxide (H_2O_2, 3%)
- Potassium iodide (KI, 0.1 M)
- Starch solution (1%)
- Black tea (2 g)
- Turmeric powder (1 g)
- Melatonin (10 mg in 100 mL water)
- Chamomile/Lavender tea (2 g)
- Ethanol (1-2%)
- Distilled water
- Beakers, test tubes, pipettes, timer

2.2. Statistical Analysis Method: P-Test

To determine the statistical significance of the antioxidant reaction times, a P-test was conducted comparing each sample's mean reaction time to the control. The null hypothesis (H_0) states that there is no significant difference between the control and antioxidant-containing samples, while the alternative hypothesis (H_1) posits that antioxidants significantly prolong the reaction time. A P-value <0.05 was considered statistically significant.

3. Results

Reaction time delays were recorded over three independent trials, with mean values calculated, as presented in Table 1. To further illustrate these differences, a histogram was generated to visually compare the reaction times of different antioxidants. The histogram (Figure 1) provides insight into the relative antioxidant effectiveness of each sample, highlighting variations in their capacity to delay oxidation.

Table 1 Mean reaction time delays (in seconds) for different antioxidant samples, recorded over three independent trials

Sample	**Average Time (s)**	**P-Value**
Control	0.01	-
Black Tea	612.47	<0.001 (significant)
Turmeric	200.56	0.034 (significant)
Melatonin	344.75	<0.005 (significant)
Chamomile/Lavender Tea	248.98	0.029 (significant)

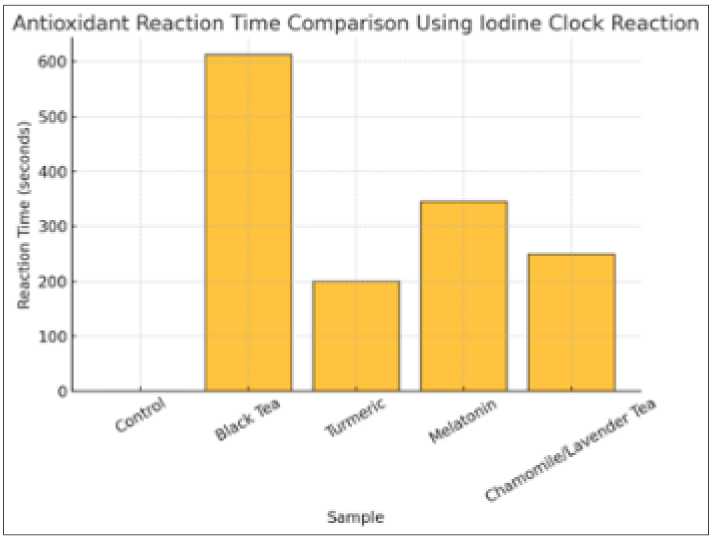

Figure 1 Histogram representation of mean reaction time delays for different antioxidants, recorded over three independent trials. The visual comparison highlights differences in antioxidant effectiveness

4. Discussion

The effectiveness of an antioxidant is directly related to the time it takes to neutralize an oxidizing agent, which can be demonstrated using the iodine clock experiment. In this reaction, iodide ions (I^-) are oxidized to iodine (I_2) by an oxidizing agent, and the appearance of a blue color indicates the formation of iodine-starch complex. When an antioxidant is introduced, it competes with iodide for the oxidizing agent, delaying the production of I_2. A stronger antioxidant reacts more quickly with the oxidizing agent, effectively postponing the iodine formation and extending the reaction time. Conversely, a weaker antioxidant allows iodine to form sooner, resulting in a shorter delay. By measuring the time until the blue color appears, the iodine clock experiment provides a clear, quantitative method for comparing antioxidant strength, demonstrating that the best antioxidants significantly prolong the reaction time by efficiently neutralizing oxidizing agents before they can oxidize iodide.

Antioxidants in the body work by neutralizing free radicals, which are unstable molecules with unpaired electrons that can cause oxidative damage to cells. Free radicals try to steal electrons from biomolecules like DNA, proteins, and lipids, leading to cellular damage and aging. Antioxidants act as electron donors, stabilizing these radicals without becoming reactive themselves. For example, Vitamin C donates an electron to a free radical, converting it into a harmless molecule while itself turning into a stable compound. Some antioxidants, like Vitamin C and Vitamin E, can even regenerate each other, enhancing their protective effect. This mechanism helps prevent oxidative stress, reducing the risk of diseases such as cancer and cardiovascular disorders while supporting overall health. Black tea is a good antioxidant because it contains polyphenols, specifically a type called flavonoids. The main antioxidants in black tea are theaflavins and thearubigins.

From a chemistry perspective, these molecules work as antioxidants because they have hydroxyl (-OH) groups attached to their rings, which can easily donate electrons to neutralize harmful oxidizing agents (like free radicals). This prevents oxidation and protects cells from damage.

In simple terms, theaflavins and thearubigins in black tea act like chemical "shields," stopping harmful oxidation reactions by giving up electrons before damage can occur. This makes black tea a strong natural antioxidant source. (Halliwell & Gutteridge, 2015; Valko et al., 2007). (Scalbert et al., 2005; Yang et al., 2018).

The results indicate significant differences in antioxidant effectiveness, with black tea showing the highest oxidative delay, consistent with literature reports on its high polyphenol content (Sahoo et al., 2014; Yang et al., 2018). Melatonin, a well-known antioxidant, ranked second, demonstrating its ability to delay oxidation effectively (Reiter et al., 2003). Chamomile/lavender tea provided moderate antioxidant activity, while turmeric exhibited the least effectiveness, possibly due to solubility issues affecting curcumin bioavailability (Anand et al., 2007). (Anand et al., 2007)

5. Conclusion

This study demonstrates that black tea exhibits the highest antioxidant potential among tested samples, with implications for dietary recommendations. Given the novelty of applying the iodine clock reaction to antioxidant assessment, further research could explore additional natural antioxidants and optimize conditions for enhanced solubility and reactivity.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest related to this research, authorship, or publication of this article.

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