

From Leaf to Line: A Quantitative Study of Spinach (*Spinacia oleracea* L.) Extract as Paper Highlighter

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Index Terms:

spinach extract, natural highlighter, sustainability, green chemistry, chlorophyll pigment, eco-friendly ink, color visibility, color retention, smear resistance, paper highlighter

Abstract. Spinach (*Spinacia oleracea* L.) contains natural chlorophyll pigments that can be utilized as an environmentally friendly dye for paper-based applications. Due to concerns about the environmental impact of synthetic fluorescent dyes and potential health risks associated with petroleum-based stationery products, plant-based extracts are being explored as sustainable alternatives for highlighter ink production. This study investigated the effectiveness of spinach extract as a natural paper highlighter by examining five dependent variables: color visibility measured through CIE Lab colorimetric analysis, color retention over 1, 2, 3, and 24 hours under ambient conditions, smear resistance using a five-point rating scale, color stability under exposure to seven visible light wavelengths (ROYGBIV), and production cost efficiency. The independent variable consisted of five formulations with varying spinach masses (50 g, 150 g, and 250 g) and acid concentrations (1.5 g and 3 g each of citric and ascorbic acid) as stabilizing agents. The study employed a true experimental quantitative design using a Pretest-Posttest Control Group Design conducted at the Maguikay National High School Science Laboratory, where spinach extract highlighters were compared with a commercial synthetic highlighter as the control group. One-way ANOVA revealed significant differences among formulations in color visibility and color retention. Formulation E (250 g spinach with 1.5 g each of citric and ascorbic acid) demonstrated the most balanced performance, achieving visibility comparable to the commercial highlighter, high color retention, strong smear resistance, good light stability, and the lowest production cost (₱21.00 per unit), indicating that spinach extract highlighters represent a viable, sustainable, and cost-efficient alternative to synthetic paper highlighters.

Introduction

Spinach (*Spinacia oleracea* L.) is a leafy green vegetable that belongs to the Amaranthaceae family and is native to central and western Asia. According to Hussin (2021), spinach extract can be used to prepare an environmentally friendly green dye suitable for highlighter ink. Through solvent extraction, the spinach dye exhibits strong green pigmentation that falls within the visible light region, making it effective for highlighting text. Its optical and morphological properties, such as clear absorption and fine particle size, allow for smooth and visible application on paper. The extended absorption range of the dye enhances its brightness and performance. As a natural alternative to toxic chemical dyes, spinach extract offers a safer and more sustainable option for producing highlighter ink while reducing environmental harm. On the other hand, a natural paper highlighter is a highlighting instrument that uses naturally derived pigments, such as botanical extracts or mineral-based colorants and biodegradable binders, to mark text on paper surfaces. Unlike conventional highlighters that rely on synthetic fluorescent dyes and petroleum-based components, this alternative emphasizes the use of renewable and low-toxicity ingredients (Pflieger & Lacoste, 2019). The development of natural paper

highlighters is part of a broader effort to reduce the environmental impact associated with disposable stationery products. Additionally, these highlighters aim to minimize potential health risks from inhalation or skin contact with harmful chemicals, making them safer for consumers. In this study, the natural paper highlighter incorporates pigment extracted from spinach, aligning sustainability goals with practical usability in highlighting printed materials.

Researchers have empirically observed significant waste issues in educational institutions, with millions of plastic highlighters being disposed of annually in landfills. According to the Environmental Protection Agency (2023), over 150 million plastic pens and highlighters are discarded each year in the United States alone, with most containing non-biodegradable components and toxic chemical residues. In the Philippines, the Department of Environment and Natural Resources (2024) reported that school supplies, including highlighters and markers, contribute substantially to plastic waste in major cities, including Cebu. The production of synthetic highlighters involves petroleum-based plastics and fluorescent dyes that persist in the environment for centuries. Proactive measures are being taken, with the Department of Education promoting the use of eco-friendly materials in schools (Virador, 2024). Concurrently, spinach production is substantial in the Philippines, with Central Visayas accounting for significant vegetable crop production (psa.gov, 2023). These efforts highlight the potential to convert agricultural waste and substandard spinach leaves into valuable resources, advancing the move towards a sustainable, circular economy in educational institutions.

Internationally, a study by Lorenzetti et al. (2021) investigated the preparation of natural fluorescent extracts from turmeric (*Curcuma longa*) using green solvents. This research was driven by the need for sustainable alternatives to synthetic fluorescent dyes, which pose environmental and health risks due to their chemical composition and persistence in ecosystems. The study found that the extracts showed bright yellow fluorescence under UV light, strong color intensity on paper substrates, and good stability under ambient conditions. Analytical assays confirmed consistent curcuminoid composition across batches, demonstrating that plant-based extracts can serve as sustainable fluorescent markers and eco-friendly alternatives to synthetic dyes. The findings revealed that natural extracts maintain functional performance while significantly reducing environmental toxicity.

Nationally, a study by Benoya et al. (2025) examined Malabar spinach (*Basella alba*) seeds as a sustainable ink source for marker applications. Using dark purple pigmentation extracted from the seeds, the researchers evaluated the ink for drying time, adhesion, smudge resistance, and application quality. The findings showed that the natural ink demonstrated comparable performance to commercial inks in all parameters tested, with shelf-life testing confirming ink stability over several weeks. Statistical analysis validated its viability as an eco-friendly alternative to petroleum-based inks. This study highlights the potential of locally available plant sources in creating sustainable writing instruments.

Locally, a study by Espartero (2023) investigated the potential of mango (*Mangifera indica*) and caimito (*Chrysophyllum cainito*) leaf extracts as natural ink substitutes for ballpoint pens. This research was conducted in response to growing environmental concerns regarding the disposal of synthetic ink cartridges and pens in Cebu's educational institutions. The study assessed the extracted ink based on color intensity, absorption on paper, and odor acceptability. Results revealed that both mango and caimito extracts produced legible and consistent ink marks, with mango demonstrating superior color quality. The experiment confirmed that natural pigments from common Philippine plants can be repurposed for writing applications, supporting the development of eco-friendly and sustainable alternatives to commercial ink products, especially in local academic contexts.

A significant gap exists in the study of spinach extract as a natural paper highlighter. Despite the proven potential of plant-based pigments in various applications, the specific use of spinach (*Spinacia oleracea* L.) extract for paper highlighting remains underexplored. This is particularly evident in the lack of localized research in areas such as Mandaue City, Cebu. There is a particular scarcity of data on how different mixing ratios of spinach mass and acid concentration affect critical ink properties such as color visibility, retention, and smear resistance. In addition, limited studies have examined how formulation factors, including stabilizer content, pigment concentration, and extraction methods, influence the performance of natural highlighters in terms of brightness, color stability under light exposure, and cost efficiency. This research aims to address these deficiencies by providing a localized investigation using accessible, small-scale extraction methods. By testing the performance of spinach extract highlighters made from different formulations, this study seeks to fill this critical gap and provide valuable data for the development of sustainable writing instruments in academic communities.

Thus, this study aimed to conduct an experimental investigation into the performance of spinach extract as a natural paper highlighter. The overall purpose of this study was to create an affordable and eco-friendly alternative to synthetic highlighters from agricultural waste, thereby supporting Sustainable Development Goal 12 (Responsible Consumption and Production).

Theoretical Background

Guided by Anastas and Warner's Green Chemistry Theory (2000) and Wake's Adsorption Theory of Adhesion (1978), this study conceptualizes the development of natural highlighters as an integrated sustainable solution aligned with Sustainable Development Goal 12. Green Chemistry Theory provides the foundational framework for designing safer chemical products using renewable, biodegradable, and non-toxic ingredients, while the Adsorption Theory explains the molecular interactions between the spinach pigment and paper fibers that influence color retention and smear resistance.

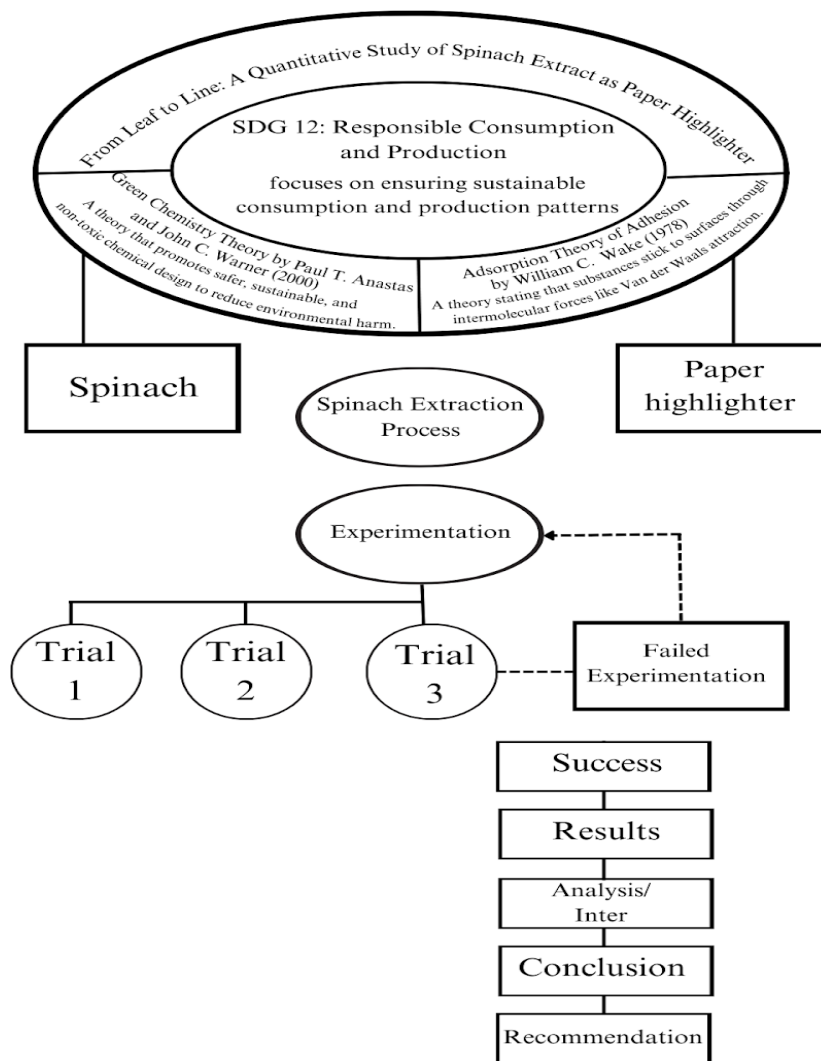


Figure 1. Schematic Diagram of Spinach Extract Production as Paper Highlighter

Statement of the problem

This study investigated the effectiveness of Spinach (*Spinacia oleracea* L.) extract as a natural paper highlighter in terms of color visibility, color retention, smear resistance, brightness, cost, and resistance to environmental factors such as light exposure and oxidation. Specifically, this study sought to answer the following questions:

Specifically, this answered the following questions;

1. What is the visibility of the Spinach (*Spinacia oleracea* L.) extract when used as a paper highlighter compared to commercial highlighters, using a colorimeter in measuring the CIE Lab in the following ratio;

- 1.1. 50 grams of spinach + 1.5 grams citric of acid powder + 0.75 grams of ascorbic powder + 20 ml of water;
 - 1.2. 50 grams of spinach + 3 grams of citric acid powder + 1.5 grams of ascorbic powder + 20 ml of water;
 - 1.3. 150 grams of spinach + 1.5 grams of citric acid powder + 0.75 ascorbic powder + 40 ml of water;
 - 1.4. 150 grams of spinach + 3 grams of citric acid powder + 1.5 grams of ascorbic powder + 40 ml of water; and
 - 1.5. 250 grams of spinach + 1.5 grams of citric acid powder + 0.75 grams of ascorbic powder + 60 ml of water?
2. Is there a significant difference in the mean L^* values (color visibility) among the five experimental groups and the commercial highlighter when used as paper highlighters?
 3. What are the experimental results comparing the spinach extract highlighter's performance against a commercially available synthetic highlighter in terms of;
 - 3.1. color retention within;
 - 3.1.1. 1 hour;
 - 3.1.2. 2 hours;
 - 3.1.3. 3 hours; and
 - 3.1.4. 24 hours?
 - 3.2. smear resistance?
 4. Is there a significant difference in the mean L^* values (color retention) among the five experimental groups and the commercial highlighter when exposed to ambient conditions over time?
 5. How does the color of the spinach extract highlighter change over time when exposed to;
 - 5.1. light, observed using ROYGBIV reference and measured using a colorimeter:
 - 5.1.1. red;
 - 5.1.2. orange;
 - 5.1.3. yellow;
 - 5.1.4. green;
 - 5.1.5. blue;
 - 5.1.6. indigo; and
 - 5.1.7. violet?
 6. What is the cost of producing a spinach extract highlighter compared to the market price of commercial highlighters?
 7. Based on the findings, what recommendations may be proposed?

Methodology

Research Design

The study employed a true experimental quantitative research design, specifically a Pretest-Posttest Control Group Design. To investigate the effectiveness of spinach (*Spinacia oleracea* L.) extract as a natural paper highlighter, this design allowed the researchers to establish cause-and-effect relationships by manipulating independent variables and observing their effects on dependent variables (Zubair, 2020). Specifically, a Pretest-Posttest Control Group Design was applied, which enabled the researchers to measure initial highlighting performance (pretest), apply interventions such as time or light exposure, and then measure outcomes again (posttest), making it easier to compare changes between the spinach extract and the commercial highlighter (control group). By integrating a true experimental design with a control group approach, the study provided a rigorous framework for systematically evaluating the effectiveness of spinach extract as an alternative natural paper highlighter.

Research Environment

The study was conducted at Maguikay National High School, specifically within the Science Department's laboratory facilities located in Sudlon, Maguikay, Mandaue City, Cebu. This specific location was chosen for two primary reasons essential to the researchers' study. First, the site provided access to essential analytical tools such as light exposure chambers, drying racks, and controlled storage containers, which were vital for evaluating the spinach extract's performance as a highlighter. The laboratory environment maintained consistent conditions of lighting, temperature, and humidity to ensure the reliability of results during testing and storage. Furthermore, the location offered logistical advantages crucial to the project's execution, as selected areas within the lab were designated for extraction, filtration, and application procedures, enabling an organized workflow and minimizing contamination throughout the duration of the study.

Research Procedures

The researchers used appropriate personal protective equipment (PPE) to prevent potential accidents and ensured safety during the experimentation. All researchers wore appropriate personal protective equipment, including surgical face masks

and gloves, throughout the process. Work surfaces were kept clean and organized to prevent mixing of samples or contamination, and materials were handled carefully to avoid accidental spills or unwanted marks on clothes, skin, and work surfaces. Testing of spinach extract highlighters was conducted in a safe, controlled environment, ensuring that any materials used did not harm the researchers or individuals nearby.

Preparation of Materials

The researchers obtained the necessary materials for the experiment from several sources. First, 1.7 kilograms of fresh spinach leaves of similar maturity were obtained from Mandaue City Public Market. Second, other essential equipment and supplies, including the blender, strainer, bond paper, distilled water, commercial highlighter, gloves, and face masks, were acquired from UNITOP. Empty highlighter containers were purchased from National Book Store, SM City J Mall. Lastly, the colorimeter was procured through Shopee due to its limited availability in local retail stores. Citric acid and ascorbic acid were purchased from local suppliers to ensure adequacy and appropriateness for the experimental procedures.

Experimental Procedures

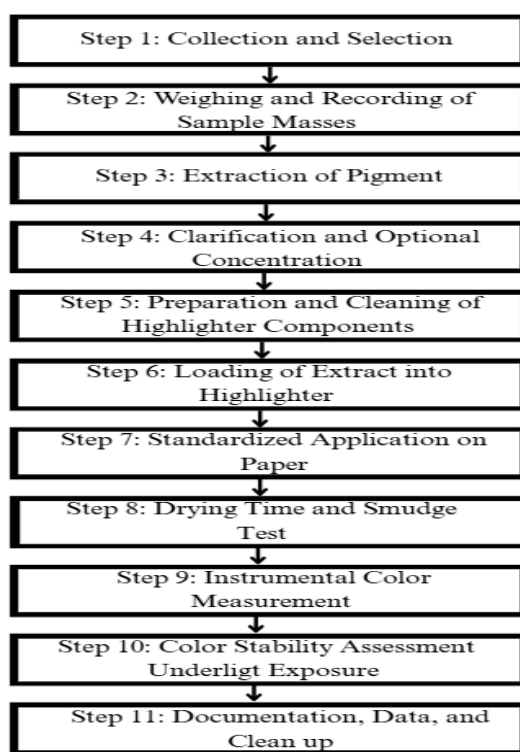


Figure 2. Flowchart of the Steps in Creating Spinach Extract Highlighter

Step 1: Collection and Selection of Spinach Leaves

The researchers obtained 1.7 kilograms of fresh spinach leaves of similar maturity from a local market and visually inspected them to remove any wilted or discolored material. Five hundred grams were allocated to each trial. The allocated leaves were rinsed under running tap water to remove dirt and surface contaminants, then air-dried on sterile absorbent paper for ten minutes prior to extraction.

Step 2: Weighing and Recording Sample Masses

Each 500-gram portion of spinach assigned to Trial 1, Trial 2, Trial 3, Trial 4, and Trial 5 was weighed using a calibrated analytical balance, and the exact mass was recorded in the laboratory notebook along with the date and sample identification code to ensure traceability across replicates.

Step 3: Extraction of Pigment

For each trial, the measured spinach leaves were placed in a sterile blender with the corresponding amount of distilled water based on the trial formulation. The mixture was blended at medium speed for two minutes until a smooth liquid was formed. The liquid was first passed through a fine mesh strainer to remove large particles, then filtered through filter paper into a sterile beaker. The resulting liquid contained the spinach's natural pigment, which served as the primary material for further processing.

Step 4: Clarification and Optional Concentration

The collected filtrate was allowed to settle for ten minutes to let any solid particles sink to the bottom. The clearer liquid was carefully poured into a second clean container, leaving the sediment behind. If the color of the extract appeared pale, the researchers gently heated it over low flame while stirring occasionally until the liquid volume was reduced by about one-fourth, making the color more concentrated. The time and appearance of the extract before and after heating were recorded.

Step 5: Addition of Stabilizing Agents

Following the specific formulation for each trial, the corresponding amounts of citric acid powder and ascorbic acid powder were added to the extract. The mixture was stirred thoroughly to ensure complete dissolution of the stabilizing agents. Citric acid and ascorbic acid were applied in equal proportions and were treated as a single stabilizing system intended to control pigment degradation and odor rather than as separate independent variables.

Step 6: Preparation and Cleaning of Highlighter Components

A brand-new, unused pen-type highlighter casing was disassembled into the barrel, reservoir sponge, and tip. Although free from any previous ink, the components were rinsed with distilled water to ensure cleanliness, then air-dried in a dust-free environment. This preparation ensured that no foreign substances interfered with the absorption or application of the spinach pigment extract.

Step 7: Loading of Extract into Highlighter

With the highlighter fully assembled, the spinach extract was introduced directly into the barrel using a funnel. The extract was dispensed slowly to allow the internal reservoir sponge to absorb it evenly. The highlighter was kept upright for five minutes to ensure complete saturation before being capped and labeled with its corresponding trial identification.

Step 8: Standardized Application on Paper

For each trial, the filled highlighter was used to apply three standard strokes across preprinted black text on white bond paper. Each stroke was performed at a consistent speed of approximately two seconds and under the same hand pressure. Ten replicate strips were prepared per trial to allow averaging of measurements.

Step 9: Drying Time and Smudge Test

Drying time was measured with a stopwatch beginning immediately after each stroke, and a light fingertip contact test was performed every ten seconds until no transfer was observed. Smudge resistance was recorded as pass or fail, and drying times for all replicates were tabulated.

Step 10: Instrumental Color Measurement

After drying, a calibrated colorimeter was used to record the CIELAB color coordinates (L , a , b) for each highlighted area, where L represented lightness, a^* represented the red-green axis, and b^* represented the yellow-blue axis. Three readings were taken per stroke and averaged to produce a color intensity value for each sample. The instrument was recalibrated between trial sets if necessary to maintain accuracy.

Step 11: Color Stability Assessment Under Light Exposure

The filled highlighter samples were exposed to controlled light sources representing the seven visible spectrum colors (ROYGBIV). For each color condition, highlighted paper samples were placed under the corresponding light for a

predetermined duration. At scheduled intervals, changes in the marked areas were observed visually and quantitatively measured using the calibrated colorimeter. This procedure determined the extent and rate of color change in the spinach extract over time under different wavelengths of light.

Step 12: Documentation, Data Storage, and Cleanup

All procedural details, including masses, volumes, blending times, evaporation durations, filter type, colorimeter readings, drying times, photographs, and deviations from protocol, were recorded in the research logbook and digital storage. Used plant pulp was disposed of according to school waste procedures, and all glassware and equipment were sanitized and stored.

Data Gathering Procedure

Formulation of the Experimental Setups

The researchers experimented at Maguikay National High School Science Laboratory, V. Albano Street, Sudlon, Maguikay, Mandaue City, Cebu. Fresh spinach leaves were collected, washed, and prepared according to their designated formulations. The materials were prepared according to their designated proportions: 50g, 150g, or 250g of spinach, with corresponding amounts of distilled water (20mL, 40mL, or 60mL). After extraction and filtration, the spinach extract was supplemented with citric acid powder and ascorbic acid powder (1.5g or 3g each) to enhance pigment stability and minimize odor. The mixture was then loaded into clean, empty highlighter containers. All highlighters were tested for color visibility, color retention, smear resistance, color stability under light exposure, and cost efficiency.

Experimental Setups

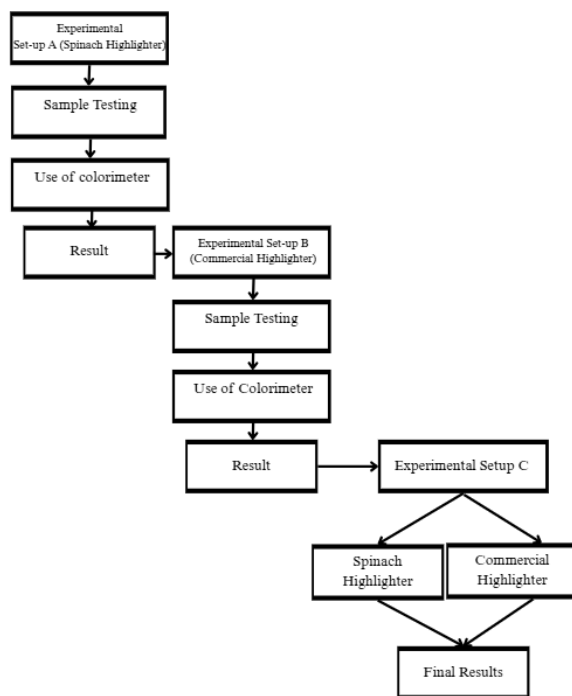


Figure 3. Flowchart of the Experimental Procedure in Testing Spinach Extract as a Natural Paper Highlighter

Experimental Setup A

Highlighters were made from 50 grams of spinach, 1.5 grams of citric acid powder, 0.75 grams of ascorbic acid powder, and 20 mL of water. This setup aimed to determine the color visibility and performance of highlighters with low spinach mass and low stabilizer concentration.

Experimental Setup B

Highlighters were made from 50 grams of spinach, 3 grams of citric acid powder, 1.5 grams of ascorbic acid powder, and 20 mL of water. This setup aimed to determine the color visibility and performance of highlighters with low spinach mass but high stabilizer concentration.

Experimental Setup C

Highlighters were made from 150 grams of spinach, 1.5 grams of citric acid powder, 0.75 grams of ascorbic acid powder, and 40 mL of water. This setup aimed to determine the color visibility and performance of highlighters with medium spinach mass and low stabilizer concentration.

Experimental Setup D

Highlighters were made from 150 grams of spinach, 3 grams of citric acid powder, 1.5 grams of ascorbic acid powder, and 40 mL of water. This setup aimed to determine the color visibility and performance of highlighters with medium spinach mass and high stabilizer concentration.

Experimental Setup E

Highlighters were made from 250 grams of spinach, 1.5 grams of citric acid powder, 0.75 grams of ascorbic acid powder, and 60 mL of water. This setup aimed to determine the color visibility and performance of highlighters with high spinach mass and low stabilizer concentration.

Positive Control

A commercial highlighter served as the positive control for this study. This allowed the researchers to compare the performance of the spinach-based highlighter against a known and established product. The commercial highlighter was tested under the same conditions as the spinach highlighter to evaluate visibility, color retention, and smear resistance.

Trial 1

In the first trial, five formulation variations were tested: (A) 50g spinach + 1.5g citric acid + 0.75g ascorbic acid + 20mL water; (B) 50g spinach + 3g citric acid + 1.5g ascorbic acid + 20mL water; (C) 150g spinach + 1.5g citric acid + 0.75g ascorbic acid + 40mL water; (D) 150g spinach + 3g citric acid + 1.5g ascorbic acid + 40mL water; and (E) 250g spinach + 1.5g citric acid + 0.75g ascorbic acid + 60mL water. Each mixture was stirred until uniform and then loaded into clean highlighter containers. The highlighters were used to mark standard bond paper and were tested for color visibility, drying time, and smear resistance. All results were recorded. If the first trial was unsuccessful, the experiment continued to a second trial using the same procedure with necessary adjustments.

Trial 2

In the second trial, the same raw material ratios and formulations were used as in the first trial. This phase focused on determining the color characteristics of the spinach highlighters, including color visibility, color retention, and smear resistance. Adjustments were made based on issues encountered during the first trial, such as inconsistent color intensity, rapid fading, or measurement inconsistencies. The testing order was randomized to reduce environmental bias, and ambient conditions, including lighting, temperature, and humidity, were documented to account for possible external effects on color performance. Results from the second trial were directly compared with those from the first to assess repeatability and precision.

Trial 3

In the third trial, the procedures from the previous trials were followed, with refinements based on the results of the second trial. These included optimizing extraction time, improving mixing uniformity, and enhancing handling techniques to prevent contamination. The same formulations were maintained across all setups. Measurements for color visibility, color retention, and smear resistance were recorded. In this trial, the spinach highlighters were also compared with commercial highlighters to evaluate performance differences. Photographs were taken before and after testing, and all data from the three trials were statistically analyzed to identify trends, ensure consistency, and validate the research findings.

Failed Trial Protocol

A trial was classified as failed if the highlighter produced no visible color, the ink was too pale to read, the extract separated or spoiled, the ink clogged the applicator tip, essential measurement data were lost, safety hazards occurred, or the extract developed foul odor. Failures were documented, causes identified, and corrected replicates produced. If the same failure occurred twice, the replicate was replaced entirely. Persistent failures were discussed with the research adviser, and necessary adjustments were implemented. Only valid replicates were analyzed, and all deviations were noted in the limitations section.

Experimentation Testing

Materials were utilized at Maguikay National High School Science Laboratory, V. Albano Street, Sudlon, Maguikay, Mandaue City, Cebu. The experimentation involved using spinach leaves as the primary raw material for natural highlighter ink, with distilled water as solvent. Five setups were prepared with varying formulations as described. Citric acid and ascorbic acid were added to stabilize the pigment and prevent oxidation. The spinach leaves were washed and prepared before extraction, then blended and filtered to obtain the pigment. Each extract was loaded into clean highlighter containers. Weighing scales, measuring cylinders, mixing containers, stirring tools, timers, colorimeters, and LED lights were used throughout preparation and testing. Three consecutive trials were conducted to ensure reliable results.

Spinach Extract Highlighter

The spinach extract highlighters were tested in three consecutive trials for each setup, all conducted in the same laboratory location to ensure uniform environmental conditions. This resulted in a total of 15 samples (5 formulations × 3 trials). Each trial involved preparing the materials, applying the highlighter to paper, and recording data on color visibility, color retention, smear resistance, and color stability under light exposure. Since the study focused on color performance and stability, the researchers recorded colorimeter readings at fixed time points throughout the testing period. A total of 6 researchers participated: 1 monitored the application consistency, 1 recorded color measurements, 1 documented observations and environmental conditions, and 3 applied the highlighter and conducted smear tests. The highlighters were used immediately after preparation to ensure freshness and prevent pigment degradation before testing.

Statistical Treatment

Hypothesis testing was employed as the primary statistical method in this study. According to DeLorey and Conroy (2025), hypothesis testing is a formal procedure for evaluating assumptions about a population parameter by analyzing sample data. It involves stating a null hypothesis (H_0) representing no effect or no difference, and an alternative hypothesis (H_1) representing the presence of an effect or difference. In this study, hypothesis testing was used to assess whether the mean performance measures of the spinach extract highlighter—including visibility, color retention, smear resistance, and color stability under light exposure—significantly differed from those of the commercial highlighter. The process involved setting the significance level at $\alpha = 0.05$, computing the appropriate test statistic from the sample data, and comparing the result to the critical value or p-value to determine whether to reject or fail to reject the null hypothesis.

A one-way analysis of variance (ANOVA) is a statistical method that compares the means of three or more independent groups to determine whether significant differences exist, by analyzing within-group and between-group variability (Chatzi & Doody, 2024). For problems 1, 2, 3.1, and 4, One-Way ANOVA was utilized to determine significant differences in color visibility and retention among the five spinach formulations and the commercial highlighter. Post-hoc tests, conducted after a significant ANOVA, identify which group means differ, providing detailed pairwise comparisons to clarify the source of the overall significance (Agbangba et al., 2024). For problems 3.2, 5, and 6, Descriptive Statistics were utilized to summarize the smear resistance ratings, color stability under ROYGBIV light exposure, and production cost analysis of each spinach formulation compared to the commercial highlighter.

Results and Discussion

The experiment utilized a colorimeter to measure the CIELAB L^* values of five spinach extract formulations and a commercial highlighter. Each formulation was applied to standard bond paper under controlled laboratory conditions at Maguikay National High School Science Department.

The experimental procedure consisted of three trials conducted to ensure reliability and consistency of results across all formulations. These trials were performed to evaluate color visibility performance and establish baseline data for comparative analysis.

	Setup A	Setup B	Setup C	Setup D	Setup E	CH
Trial 1	30.67 L*	39.36 L*	48.94 L*	56.64 L*	72.01 L*	85.23 L*
Trial 2	36.20 L*	44.52 L*	57.02 L*	63.23 L*	78.23 L*	85.35 L*
Trial 3	41.23 L*	55.39 L*	64.50 L*	73.34 L*	81.01 L*	85.49 L*
Average	36.03 L*	46.42 L*	56.82 L*	64.40 L*	77.08 L*	85.36 L*

Table 4. Color Lightness (L Value) of the Different Setups.*

Table 4 presents the color lightness (L* value) measurements of each highlighter setup across three trials, where higher values indicate greater brightness and visibility. Setup E exhibited the highest mean L* value among spinach formulations, indicating that increased spinach mass combined with citric and ascorbic acid enhanced chlorophyll concentration and light reflection; in contrast, Setup A recorded the lowest mean L* value due to limited pigment concentration, while the Commercial Highlighter demonstrated the highest overall mean L* value attributable to synthetic fluorescent dyes designed to maximize contrast. Hussin (2021) supports these findings, explaining that chlorophyll-based pigment brightness depends on concentration and stabilization, aligning with Setup E's superior performance among natural formulations. Singh et al. (2022) confirmed that natural inks produce legible markings but lack the brightness of synthetic inks due to absent fluorescent compounds, while Birriel and King (2018) emphasized that fluorescent agents enhance brightness, reinforcing the commercial highlighter's highest L* value. Overall, the findings demonstrate that spinach extract has potential as a natural highlighter at higher pigment concentrations, though further formulation enhancement is needed to match synthetic brightness, confirming that pigment concentration and chemical stabilization are crucial determinants of highlighter effectiveness.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	5	5150.3616	1030.0723	24.9792	0.000005917
Error (within groups)	12	494.8459	41.2372		
Total	17	5645.2075	332.071		

**significant at p-value < 0.05*

Table 5. The significant difference in color lightness (L value) among the different highlighter setups.

Table 5 presents the one-way ANOVA results for color lightness (L* value), revealing a statistically significant difference among groups (F = 24.9792, p = 0.000005917), with a between-groups mean square of 1,030.07 compared to within-groups mean square of 41.24. These results indicate that observed variations in mean L* values are extremely unlikely due to chance, leading to rejection of the null hypothesis (H₀₁) and confirming significant differences in color visibility among spinach extract setups and the commercial highlighter. To identify specific group differences while controlling Type I error, Tukey's HSD post-hoc analysis was conducted.

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	10.39	3.7075	2.8024	-7.2216	28.0016	17.6116	0.4046
x1-x3	20.7867	3.7075	5.6066	3.1751	38.3983	17.6116	0.018
x1-x4	28.37	3.7075	7.652	10.7584	45.9816	17.6116	0.001683
x1-x5	41.05	3.7075	11.0721	23.4384	58.6616	17.6116	0.00005375
x1-x6	49.3233	3.7075	13.3036	31.7117	66.9349	17.6116	0.000008077
x2-x3	10.3967	3.7075	2.8042	-7.2149	28.0083	17.6116	0.404
x2-x4	17.98	3.7075	4.8496	0.3684	35.5916	17.6116	0.04444
x2-x5	30.66	3.7075	8.2697	13.0484	48.2716	17.6116	0.0008577
x2-x6	38.9333	3.7075	10.5012	21.3217	56.5449	17.6116	0.00009103

x3-x4	7.5833	3.7075	2.0454	-10.0283	25.1949	17.6116	0.7011
x3-x5	20.2633	3.7075	5.4655	2.6517	37.8749	17.6116	0.02131
x3-x6	28.5367	3.7075	7.697	10.9251	46.1483	17.6116	0.001601
x4-x5	12.68	3.7075	3.4201	-4.9316	30.2916	17.6116	0.2239
x4-x6	20.9533	3.7075	5.6516	3.3417	38.5649	17.6116	0.01706
x5-x6	8.2733	3.7075	2.2315	-9.3383	25.8849	17.6116	0.6261

*significant at p-value < 0.05; x1 – Setup A, x2 – Setup B, x3 – Setup C, x4 – Setup D and x5 – Setup E and x6 – Commercial Highlighter

Table 6. Tukey's Honestly Significant Difference (HSD) for Color Visibility

Table 6 presents the Tukey HSD post-hoc pairwise comparison results for color lightness (L* value), revealing that Setup E showed no statistically significant difference from the commercial highlighter (p = 0.6261), indicating comparable brightness and visibility, while Setups A, B, C, and D showed significant differences (p < 0.05), with Setup A exhibiting the largest difference (p = 0.000008077). The absence of significant differences between adjacent higher-mass formulations (C vs D, p = 0.7011; D vs E, p = 0.2239) indicates that beyond a certain pigment concentration threshold, brightness improvements plateau. These findings are supported by Sengupta et al. (2025), Lombardelli et al. (2024), and Nagrale et al. (2022), who confirmed that pigment concentration directly affects optical performance, stability, and adhesion. Grounded in Green Chemistry Theory (Anastas & Warner, 1998), the statistical comparability of Setup E demonstrates that environmentally responsible materials using renewable feedstocks and natural stabilizers can achieve competitive optical performance, confirming that pigment concentration is the dominant factor influencing brightness while stabilization enhances durability, establishing the high-concentration spinach formulation as both scientifically viable and environmentally advantageous.

	Setup A	Setup B	Setup C	Setup D	Setup E	CH
1 hr	34.11 L*	44.20 L*	54.93 L*	62.55 L*	75.40 L*	85.36 L*
2 hr	32.40 L*	42.75 L*	52.18 L*	60.10 L*	73.22 L*	85.35 L*
3 hr	30.85 L*	40.90 L*	50.44 L*	58.38 L*	71.64 L*	85.32 L*
24 hr	29.32 L*	39.60 L*	48.44 L*	56.88 L*	70.14 L*	85.26 L*
Average	31.67 L*	41.86 L*	51.50 L*	59.48 L*	72.60 L*	85.32 L*

Table 7. Color Lightness (L Value) Retention of the Different Highlighter Setups Across Time

Table 7 presents the color lightness (L* value) retention of each highlighter setup measured at 1, 2, 3, and 24 hours of ambient exposure, showing progressive fading behavior and color stability. The Commercial Highlighter exhibited the highest and most stable brightness with a mean of 85.32 L showing negligible change, while Setup A recorded the lowest mean at 31.67 L* reflecting poor initial brightness and significant fading. Setup E demonstrated the highest mean among spinach formulations at 72.60 L, indicating that increased spinach mass and citric acid enhanced chlorophyll concentration and stability. A gradual decrease in L values across all spinach setups from 1 to 24 hours confirms chlorophyll susceptibility to light and air degradation, though Setups D and E maintained relatively higher values after 24 hours, demonstrating that higher pigment concentration and stabilizing agents improve durability. These findings are supported by Kumari and Sharma (2021) and Chungkrang et al. (2021), while Anastas and Warner's (2000) Green Chemistry Theory explains that plant-based pigments can provide functional alternatives despite performance trade-offs. Overall, the results demonstrate that spinach extract can serve as a functional natural paper highlighter at higher pigment concentrations, though further formulation improvements are necessary to enhance brightness and long-term visibility, confirming that pigment concentration, stabilization, and formulation composition are key factors influencing highlighter performance.

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Groups (between groups)	5	7810.646	1562.1292	348.5353	0.000000
Error (within groups)	18	80.6757	4.482		
Total	23	7891.3217	343.1009		

Table 8. Significant difference in color retention (L value) among highlighter setups over time.

Table 8 presents the one-way ANOVA results for color retention (L^* value) across 1, 2, 3, and 24 hours of ambient exposure, revealing a statistically significant difference among groups ($F = 348.5353$, $p = 0.000000$), with a large between-groups mean square (1,562.13) compared to within-groups mean square (4.48). These results indicate that observed variations in mean L^* values are extremely unlikely due to chance, leading to rejection of the null hypothesis (H_{02}) and confirming significant differences in color retention among spinach extract setups and the commercial highlighter over time. To identify specific group differences while controlling Type I error, Tukey's HSD post-hoc analysis was conducted.

Pair	Difference	SE	Q	Lower CI	Upper CI	Critical Mean	p-value
x1-x2	10.1925	1.0585	9.6289	5.435	14.95	4.7575	0.00002892
x1-x3	19.8275	1.0585	18.7311	15.07	24.585	4.7575	0.0000000
x1-x4	27.8075	1.0585	26.2698	23.05	32.565	4.7575	0.0000000
x1-x5	40.93	1.0585	38.6667	36.1725	45.6875	4.7575	0.0000000
x1-x6	53.6525	1.0585	50.6856	48.895	58.41	4.7575	0.0000000
x2-x3	9.635	1.0585	9.1022	4.8775	14.3925	4.7575	0.00005954
x2-x4	17.615	1.0585	16.6409	12.8575	22.3725	4.7575	0.0000000
x2-x5	30.7375	1.0585	29.0378	25.98	35.495	4.7575	0.0000000
x2-x6	43.46	1.0585	41.0568	38.7025	48.2175	4.7575	0.0000000
x3-x4	7.98	1.0585	7.5387	3.2225	12.7375	4.7575	0.0005575
x3-x5	21.1025	1.0585	19.9356	16.345	25.86	4.7575	0.0000000
x3-x6	33.825	1.0585	31.9546	29.0675	38.5825	4.7575	0.0000000
x4-x5	13.1225	1.0585	12.3969	8.365	17.88	4.7575	0.0000000
x4-x6	25.845	1.0585	24.4158	21.0875	30.6025	4.7575	0.0000000
x5-x6	12.7225	1.0585	12.019	7.965	17.48	4.7575	0.000001358

Table 9. Tukey's Honestly Significant Difference (HSD) for Color Retention

Table 9 presents the Tukey HSD post-hoc pairwise comparison results for color retention over 24 hours, revealing that the commercial highlighter has statistically significant higher color retention compared to all spinach-based setups ($p < 0.05$), while significant differences among spinach formulations showed progressive improvements, indicating that higher pigment concentration and stabilizer optimization enhance retention performance. Birriel and King (2018) support these findings by demonstrating that synthetic fluorescent dyes are engineered for superior photostability, explaining why the commercial product consistently outperformed natural formulations. Furthermore, Lombardelli et al. (2024) found that proper formulation techniques can significantly slow chlorophyll pigment degradation, validating the observed progressive improvement among spinach setups. These studies confirm that pigment composition, concentration, and stabilization methods are key factors in color retention, demonstrating that while spinach-based formulations show promising stability, commercial synthetic highlighters maintain a technological advantage due to their chemically engineered dye structures.

	Setup A	Setup B	Setup C	Setup D	Setup E	CH
Trial 1	2	3	3	4	4	4
Trial 2	2	2	3	3	4	5
Trial 3	1	3	4	4	5	5
Mean	1.67	2.67	3.33	3.67	4.33	4.67

*Legend: 5 – No smearing: Ink remains completely intact; no transfer or blurring. 4 – Slight smearing: Minimal transfer; text remains clearly legible. 3 – Moderate smearing: Noticeable blurring; text is still readable. 2 – Significant smearing: Heavy smudging; text partially obscured. 1 – Severe smearing: Ink spreads widely; text becomes illegible.

Table 10. Smear Resistance of the Different Highlighter Setups

Table 10 presents the mean smear resistance ratings of each highlighter setup across three trials using a five-point scale, where higher values indicate better resistance to smudging. Among experimental setups, Setup E obtained the highest mean rating among spinach formulations (4.33), corresponding to slight smearing with minimal ink transfer and clearly legible text, indicating that higher spinach pigment concentration improved formulation stability and reduced ink spread. In contrast, Setup A recorded the lowest mean rating (1.67), interpreted as severe smearing where ink spread widely and caused text illegibility due to insufficient pigment stability. Gradual improvements were observed across Setups B (2.67), C (3.33), and D (3.67), demonstrating that increased pigment concentration progressively enhanced smear resistance. The Commercial Highlighter achieved the highest overall mean rating (4.67), corresponding to no smearing to slight smearing, attributable to synthetic binders and fluorescent compounds designed to control ink absorption. These findings align with Kumari and Sharma (2021) and Chungkrang et al. (2021), who emphasized that higher chlorophyll concentration and formulation stability improve natural dye performance on cellulose-based materials. Guided by Anastas and Warner's (2000) Green Chemistry Theory, the findings highlight spinach extract's potential as an environmentally friendly alternative, demonstrating that higher pigment concentration significantly improves smear resistance and ink stability.

Color	Setup A	Setup B	Setup C	Setup D	Setup E	CH
Red	2	3	3	4	4	4
Orange	2	3	3	4	4	4
Yellow	3	3	4	4	5	4
Green	2	3	3	4	4	4
Blue	1	2	3	3	4	3
Indigo	1	2	2	3	3	3
Violet	1	2	2	3	3	3
Mean	1.71	2.57	2.86	3.57	3.86	3.57

**Legends: 5 – No color change, 4 – Slight change, 3 – Moderate, 2 – Large change, 1 –Very large change*

Table 11. Color Stability of the Different Highlighter Setups Under Light Exposure

Table 11 presents the mean color stability ratings of each highlighter setup when exposed to the seven visible light spectrum colors (ROYGBIV) using a five-point scale, where higher values indicate greater resistance to photodegradation. Based on this scale, Setup E obtained the highest mean rating among experimental setups (3.86), experiencing only slight color changes under light exposure due to higher spinach pigment concentration and stabilizing agents that protected chlorophyll from photodegradation, a finding supported by Hussin (2021) and Lombardelli et al. (2024). In contrast, Setup A recorded the lowest mean rating (1.71), corresponding to large color changes due to insufficient pigment concentration and weaker stabilization, aligning with Singh et al. (2022). The Commercial Highlighter obtained a high mean rating (3.57) due to synthetic fluorescent dyes designed to resist photochemical degradation, as supported by Birriel and King (2018); however, it did not surpass Setup E, suggesting that properly formulated spinach extract can achieve comparable or better color stability, consistent with Kotowicz et al. (2024). Overall, these findings indicate that pigment concentration and stabilization are crucial factors affecting color stability, with Setup E's superior performance demonstrating that natural pigments can effectively resist color change and compete with commercial highlighters, reinforcing the viability of spinach extract as a stable, eco-friendly alternative to synthetic highlighter inks.

Setups	Costs	Cost per quantity
Setup A	₱302	₱60
Setup B	₱305	₱61

Setup C	₱311	₱31
Setup D	₱317	₱32
Setup E	₱320	₱21
CH	₱200	₱33

Table 12. Production Cost Analysis of Spinach Extract Highlighter Setups and Commercial Highlighter.

Table 12 presents the production costs of each highlighter setup, showing that the Commercial Highlighter has a total cost of ₱200.00 with a retail price of ₱33.00 per unit, while among spinach formulations, Setup E recorded the lowest cost per highlighter at ₱21.00—36.4% cheaper than the commercial product—with Setups C (₱31.00) and D (₱32.00) also being slightly more affordable. In contrast, Setups A (₱60.00) and B (₱61.00) were 81.8% to 84.8% more expensive, indicating that low-concentration spinach formulations are economically inefficient despite lower total batch costs. Setup E emerged as the most cost-effective formulation, achieving the lowest production cost while demonstrating the best performance among natural formulations in visibility, retention, smear resistance, and color stability, establishing it as the optimal sustainable alternative. These findings demonstrate that increasing spinach concentration reduces per-unit cost as higher-yield batches distribute material costs across more functional units. The economic advantages are supported by Repon et al. (2019), who found natural dyeing costs approximately 50% lower than synthetic dyeing, corroborating Setup E's 36.4% cost reduction, and by Jeevanandam et al. (2020), who reported that natural pigment production costs have declined with technological advancements, positioning cost-competitive formulations like Setup E for market adoption and commercial scalability.

Conclusion and Implications

Conclusion

This study evaluated the performance and production cost of spinach extract highlighters as a natural alternative to commercial synthetic highlighters, confirming that variations in spinach mass and acid concentration significantly affected visibility, retention, smear resistance, color stability, and cost. Among the experimental setups, Setup E demonstrated the most balanced and efficient performance, achieving statistical equivalence to the commercial highlighter in visibility, superior color stability, best natural formulation performance in retention and smear resistance, and the lowest per-unit cost. These findings are supported by Anastas and Warner's Green Chemistry Theory (2000) on designing safer, renewable, and biodegradable products, and by Wake's Adsorption Theory of Adhesion (1978) on molecular bonding between ink and paper fibers affecting durability and smudge resistance. However, the inferior color retention of Setup E compared to the commercial highlighter indicates the need for further advancements in stabilization techniques to achieve complete parity with synthetic products. Ultimately, in alignment with Sustainable Development Goal 12 on Responsible Consumption and Production, this study demonstrates that spinach extract highlighters, particularly Setup E, can emerge as a viable, sustainable, economically superior, and environmentally responsible alternative to commercial synthetic highlighters.

Implication

The outcomes are consistent with Anastas and Warner's Green Chemistry Theory (2000), which explains that chemical products should be designed to reduce or eliminate hazardous substances while maintaining functionality, as well as with Wake's Adsorption Theory of Adhesion (1978), which emphasizes the importance of molecular bonding between ink and paper fibers in achieving durability and smudge resistance; however, the relatively lower color retention and performance variability observed in some setups highlight the need for improvements in stabilization techniques, pigment concentration optimization, and formulation refinement, and in line with Sustainable Development Goal 12, continued refinement and technological optimization can enable spinach extract highlighters to become a viable, sustainable, and environmentally responsible alternative to commercial synthetic highlighters.

Recommendation

Based on the findings and conclusions, the following recommendations are proposed: (1) Students may benefit from using cost-effective and eco-friendly highlighters made from natural materials like spinach extract, as these alternatives are safe, accessible, and promote awareness of environmental responsibility while enhancing creativity in schoolwork. (2) Teachers may integrate plant-based and environmentally friendly experiments into classroom activities to strengthen students'

understanding of scientific concepts, sustainability, and real-world applications through hands-on learning. (3) Environmental Advocates may utilize this study as evidence that biodegradable, plant-based materials can serve as alternatives to synthetic school supplies, helping reduce chemical waste and promote renewable resources in daily use. (4) School Administrators may support eco-friendly and budget-conscious school supplies such as natural highlighters to strengthen school sustainability programs and demonstrate environmental leadership within the educational sector. (5) Researchers may use the findings as a foundation for further investigations into plant-based inks and sustainable writing materials, particularly in improving formulation, visibility, and durability. (6) Future Researchers may build upon the methodology and experimental data presented to further examine spinach extract formulations, test alternative natural additives, and improve stabilization techniques to enhance color retention and overall performance. (7) Product Developers and Innovators may investigate the commercial scalability of spinach extract highlighters, including the development of more effective natural stabilizers, improved extraction methods, and appropriate packaging solutions that maintain product quality while minimizing environmental impact. (8) Policy Makers may integrate natural highlighter initiatives into local waste management and sustainable education programs to convert agricultural waste into renewable resources, supporting the circular economy and reducing the environmental footprint of educational institutions. (9) Local Communities and Farmers may consider spinach leaves, including those not suitable for food markets, as potential raw materials for natural highlighter production, creating supplementary income sources and promoting resource efficiency at the community level.

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Competing Interests Statement

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Data Availability Statement

Data availability can be accessed by submitting a formal letter of request to the author of the study

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Appendices

No appendices are attached in this study