



Eradequine: Atis (*Annona squamosa*) leaves and coconut milk against human head lice (*Pediculus humanus capitis*)

¹Fritzie Lorraine D. Mapang & ²Mariane Miles M. Basangal

Abstract

Head lice, or pediculosis, is a global health problem that affects people of all age groups. It poses a significant concern for individual health—particularly among children—as well as their social interactions with peers. Furthermore, commercial pediculicides, namely permethrin and pyrethroids, have been found to be decreasing in effectiveness, as human head lice in various countries and regions are developing resistance through mutation. This study investigated the pediculicidal properties of coconut milk and macerated Atis leaves in three concentrations: 50:50, 75:25, and 25:75. A true experimental design was used, and the study was conducted in triplicate via in vitro experimentation. Three head lice per treatment ratio, along with a control group, were placed in plastic containers lined with filter paper treated with the respective solutions. Based on the average time to reach mortality, the 25:75 ratio of coconut milk to Atis leaves demonstrated the fastest pediculicidal effect. An independent t-test showed a non-significant p-value when comparing Eradequine and Kwell (a commercial pediculicide). Pearson r results revealed a positive correlation between Eradequine and the mortality rate of the lice.

Keywords: head lice, atis, custard apple, coconut milk, pediculosis, pediculicide

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About the authors:

¹Corresponding author. Grade 10 student, City of San Jose del Monte National Science High School. Email: fritzielorraine.mapang@csjdmnshs.ph.education

²Grade 10 student, City of San Jose del Monte National Science High School. Email: marianemiles.basangal@csjdmnshs.ph.education



1. Introduction

Head lice is a persistent global health issue that has existed since prehistoric times. Characterized by an irritating itch on the scalp, lice infestation, or pediculosis, significantly disrupts the daily lives of those affected. In 2022, the Department of Education (DepEd) in the Philippines reported that lice infestation ranks as the second most common health concern among students, following tooth decay (Abarca, 2022). This data underscores the widespread nature of the condition in school environments. Beyond the physical discomfort, pediculosis also imposes social and psychological burdens, especially on children. Affected students may experience feelings of guilt, embarrassment, and fear of stigmatization, particularly due to the possibility of spreading lice to classmates or being perceived as lacking personal hygiene. These psychosocial consequences emphasize that lice infestations are not only health concerns but also issues that affect mental well-being and social development (Babazadeh et al., 2023).

Although commercially available pediculicides offer an immediate solution, their use raises significant environmental and pharmaceutical concerns. These chemical-based treatments are closely tied to the pharmaceutical industry and are not environmentally benign. Patneedi et al. (2015) highlight that the unregulated release of pharmaceutical compounds into ecosystems, particularly aquatic systems, can lead to serious ecological consequences. These substances, when discharged into water bodies, may alter aquatic life, disrupt food chains, and even return to human populations through contaminated water sources. This environmental risk highlights the urgent need for safer, eco-friendly alternatives to conventional lice treatments (Patneedi et al., 2015).

In addition to environmental concerns, the growing resistance of lice to chemical pediculicides is another pressing issue. In Northwestern Iran, permethrin resistance has been confirmed in head lice populations due to the presence of knockdown resistance (kdr)-type mutations, which are strongly linked to pyrethroid resistance. These mutations interfere with the voltage-sensitive sodium channel (vssc) gene, affecting the normal function of nerve cells in lice and reducing the efficacy of treatments (Ghavami et al., 2023). Furthermore, global surveillance of resistance shows that head lice populations in regions such as North and South America, Asia, the European Union, Oceania, and Africa have resistant allele frequencies ranging from 15% to 100%, making it a widespread concern (Fox et al., 2020). These findings suggest that the continued reliance on synthetic pediculicides may no longer be sustainable or effective.

In response to these issues, this study explores natural and locally available alternatives, specifically Atis seeds (*Annona squamosa*) and coconut milk. Atis seeds have been reported to possess several medicinal properties, including anti-lice activity, making them a promising natural solution. However, despite the known benefits of the seeds, the leaves of *A. squamosa* are rarely explored in this context (Safira et al., 2022). Prior studies have shown that the leaves contain high nutritional and bioactive compounds that may have health applications (Kumar et al., 2021; Sarker et al., 2024; Abdul Wahab et al., 2018; Andrade et al., 2021; Kumari et al., 2022). Nevertheless, current literature on the pediculicidal potential of Atis leaves remains limited, indicating the need for further research and scientific validation.

A. squamosa is widely accessible to Filipino households, making it a practical candidate for community-level use. It is commonly found as a backyard tree, particularly in tropical regions, and is prevalent throughout the Philippines, with the exception of parts of Mindanao (Crane et al., 2022). This makes it both economically and geographically viable for inclusion in natural health solutions. Similarly, coconut milk is another widely available resource in the Philippines. Despite its accessibility and health benefits, research by Bahad et al. (2022) has shown that coconut milk alone is not effective against lice, as it only causes temporary paralysis. Tested in concentrations of 100%, 75%, and 50%, it was found to have minimal pediculicidal efficacy (Bahad et al., 2022). This suggests that while coconut milk may serve as a complementary agent, it requires combination with other bioactive ingredients, such as Atis, to enhance effectiveness.

From an agricultural and economic standpoint, coconuts represent a major export product of the Philippines, second only to Indonesia in global production. According to the Department of Agriculture, coconut exports have consistently contributed around 35% of the country's agricultural exports, with revenues reaching USD 3.2 billion in 2022, accounting for 43% of total agricultural exports. This underscores both the economic value and abundant supply of coconuts in the country, reinforcing the practicality of coconut-based treatments.

This study is driven by the urgent need to address the growing frequency of lice infestations, the emergence of permethrin-resistant strains, and the environmental drawbacks of synthetic pediculicides. Given the local abundance and potential medicinal benefits of Atis leaves and coconut milk, these natural resources represent promising, underutilized alternatives in the search for safer, sustainable, and culturally appropriate solutions to pediculosis.

This study investigates the pediculicidal activity of coconut milk and macerated Atis leaves against adult head lice collected in the City of San Jose del Monte, Bulacan in the Philippines. The aspects examined include the total number of lice killed, the speed of action, and the effectiveness compared to commercial pediculicides.

This study tests the following hypotheses:

On the most effective ratio, there are four hypotheses tested:

H01: None of the ratios are able to reach a total mortality rate.

Ha1: A ratio of 25:75 coconut milk and atis leaves yields the fastest time in killing all the lice.

Ha2: A ratio of 75:25 coconut milk and atis leaves yields the fastest time in killing all the lice.

Ha3: A ratio of 50:50 coconut milk and atis leaves yields the fastest time in killing all the lice.

On the viability of Eradequine, there are two hypotheses tested:

H02: Eradequine is not a viable alternative to commercial pediculicides.

Ha4: Eradequine is a viable alternative to commercial pediculicides.

On the correlation between Eradequine and the mortality rate of lice, there are three hypotheses tested:

H03: There is a negative correlation between Eradequine and the mortality rate of lice.

H04: There is no correlation between Eradequine and the mortality rate of lice.

Ha5: There is a positive correlation between Eradequine and the mortality rate of lice.

2. Literature review

2.1 Lice

The Lice Clinics of America (2016) state that lice are believed to have existed 1.68 billion years before homo sapiens. Despite several years of evolution, lice still trouble more than hundreds of millions of humans every day. While not considered a health hazard, it is still an inconvenience for many. Moreover, a lice infection is medically coined as pediculosis. Head lice are characterized as parasitic insects 2-3 mm long or around the size of a sesame seed (Fried et al., 2008; Veracz & Raoult, 2012; About Head Lice, 2024). They cannot hop nor fly,

and instead crawl from one place to another, commonly by hair-to-hair contact. It is worth noting that head lice are a sub-species separate from pubic lice. Furthermore, lice carry social difficulties that are experienced by children and families afflicted by it. Such were lack of support from other family members, children's exposure to verbal and physical violence, exclusion from the school and society due to stigma, children's refusal to be treated, difficulties in the physical removal of the nits, inability to pay for the pediculicide, and inappropriate physical conditions of the house (Ozkan et al., 2012). The stigma around pediculosis commonly revolves around assumptions of poor hygiene (Banafshi & Khatony, 2024; Gray, 2022; Neuberg et al., 2022), which is irrelevant in the factors affecting the likelihood of someone getting pediculosis (Babazadeh et al., 2023).

2.2 Atis

Annona squamosa, commonly called the sugar apple or Atis in the Philippines, is a green fruit with a scaly rind and a creamy pulp that comes apart in segments with black seeds. The Philippines is a prime producer of Atis, and many Filipino citizens have it as a common backyard tree (Crane et al., 2022). A study by the Department of Environment and Natural Resources (2015) state that Atis is cultivated in the Philippines and is occasionally spontaneous. It is very likely that the Atis fruit was introduced to the Philippines by the Spaniards during the Spanish era (Chen et al., 2011). Geographically, it appears on low to medium heights. Across the tropics, it is also cultivated in the subtropical regions of Florida and is widely dispersed throughout the Philippines, except in Mindanao (Crane et al., 2022). Moreover, Atis leaves possess a variety of properties. To name few, the other uses of different sections of Atis trees include treatment for dysentery, epilepsy, hemorrhages, fevers, and tumors (Kumar et al., 2021; Al Kazman et al., 2022). However, a study conducted by Safira et al. (2022) states that the leaves of *A. squamosa* are rarely used. The leaves, fruit, and seed of Atis contain vermifugal properties, can repel or possibly exterminate insects, which may be attributed to the anthraquinones found within it (Nhung & Qoc, 2024).

A study by Benitez et al. (2015) attempted to create a pediculicidal treatment using Atis seeds. Three separate trials were conducted: namely with one control group (75 percent permethrin) and three experimental groups at doses of 25%, 50%, and 75%. The Atis seed extract was manually extracted and crushed; 30g crushed seeds were combined with 60ml of distilled water and filtered through cheesecloth. Potency is determined by its ability to interfere

with the growth of ten lice on petri dishes, leading to their death. A 15-minute observation of the number of dead lice will be conducted. Observations will continue for 30 and 45 minutes. The last observation was the killing time for one-half of the total head lice per treatment. With the permission of the kids and their parents, 120 adult head lice were collected and placed in petri dishes with 10 lice each. After an hour, the head lice were observed under a magnifying glass before applying the solutions to analyze its behavior. To check the effectiveness of the treatments, signs of death were observed; this includes no movement even with forceps stimulation. Observations were done at 15, 30, and 45 minutes, and the official time it took for half of the head lice to be officially considered dead in each treatment was recorded. The fastest solution observed was a ratio of 50%.

2.3 Coconut

Coconut is another stalwart of traditional medicine, it boasts a rich history steeped in folklore and empirical observation (DebMandal & Mandal, 2011; Lima et al., 2015; Chaachouay & Zidane, 2024), also revered as the "Tree of Life" in many cultures; being valued for their multifaceted utility, providing sustenance, shelter, and medicine to countless generations. The Philippines is the 2nd leading producer of coconuts, following Indonesia (Department of Agriculture, 2023; Lu, 2023). According to the Department of Agriculture, the coconut business has been home to the country's agricultural export champions; in recent decades, coconut exports have contributed an average of 35% of the country's overall agricultural exports and in 2022, coconut exports totaled USD 3.2 billion, accounting for 43% of the country's agricultural exports.

Coconut contains lauric acid, making it an anti-microbial, anti-protozoan, and antiviral, dietary fibers that have a hypercholesterolemic effect, and hemicellulose component that has cholesterol-lowering properties along with coconut water that has hypocholesterolemic antioxidant, and antithrombotic properties (Nevin et al., 2006; Beegum et al., 2022; Ameena et al., 2024; Mat et al., 2022). Bahad et al. (2022) conducted a study to discover the pediculicidal adverse effects of (*Cocos nucifera* L.) milk against adult infestations of head lice in individuals, using a variety of concentrations including 100%, 75%, and 50%. The study attempted to determine the most appropriate amount for obtaining the intended pediculicidal effect by methodically testing these various concentrations of the substance. It confirms the paralyzing effects of coconut milk while deeming it ineffective for killing lice.

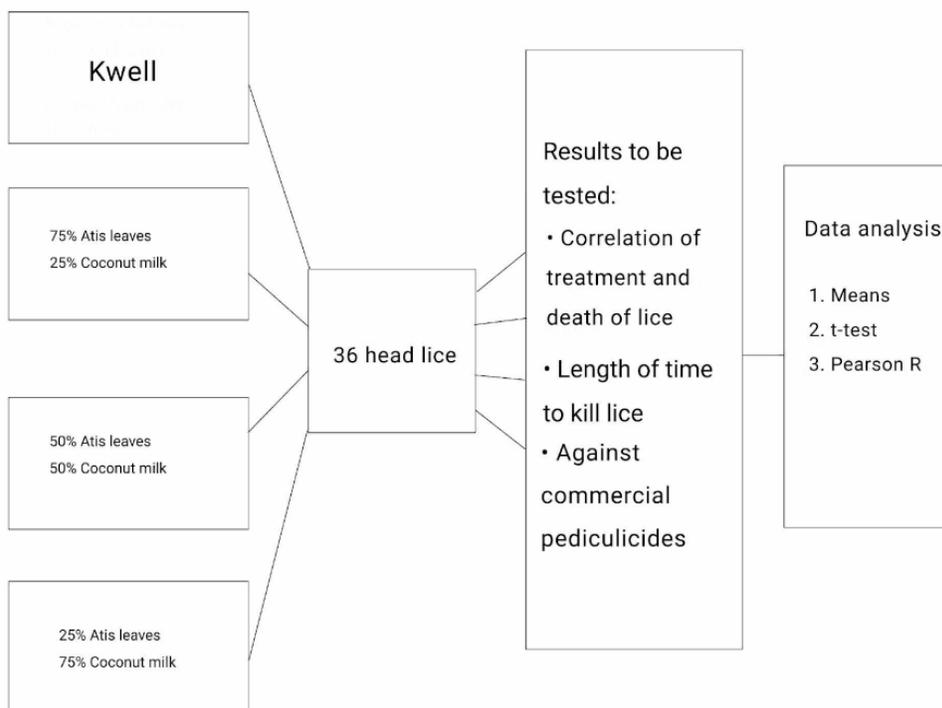
2.4 Commercially Available Pediculicides

While pharmaceutical medicine is a considerable solution, the availability of such can be a hurdle, especially in more rural provinces can be considerably scarce, or if not both, costly (Singhasivanon et al., 2019). A study by Arote et al. (2012) highlights the use of neurotoxin-based compounds, such as permethrin and pyrethrin which are common and standard treatments for head lice and warns against the possible health concerns associated with these chemicals, including neurotoxicity and anemia, especially when misused. In relation, a study by Hamad (2016), reports a child with acute organophosphate poisoning caused by the misuse of a pediculicide, specifically Diazinon-60, for head lice. Furthermore, Ghavami et al. (2023) found that the voltage-sensitive sodium channel (VSSC) gene harbors knockdown resistance (KDR) mutations highlight the importance of pyrethroid target site insensitivity as a major resistance mechanism in the treatment of pediculosis. Pyrethroids, frequently found in lice control treatments, work insecticidally by interfering with the vssc gene, preventing parasite neurons from working normally. However, the appearance of kdr mutations in lice populations suggests that these pests have undergone genetic changes that provide tolerance to the pyrethroids' neurotoxic effects. This finding presents a serious threat to established pediculosis management plans since it raises the possibility that pyrethroid-based therapies will not work against populations of resistant lice as it has already done so in many regions such as North and South America, Asia, the European Union, Oceania, and Africa (Fox et al., 2020).

The pharmaceutical industry may also cause environmental harm (Patneedi et al., 2015). Residue from manufacturers are released into rivers, streams, and soil which affect non-target organisms such as wildlife and human civilization; wildlife, when exposed to pharmaceutical residues, may undergo behavioural changes reproduction pattern disturbances, or other negative impacts (Mishra et al., 2023; Dehkordi et al., 2024; Anand et al., 2022; Okuthe et al., 2025; Eapen et al., 2024; Ortúzar et al., 2022). It is important to understand these effects in order to preserve the ecological balance and guarantee the health of various ecosystems.

3. Methodology

This study used four (4) treatments utilizing a true-experimental design and in-vitro testing. This study aimed to assess the pediculicidal effect of coconut milk and crushed atis leaves. The trials were done in triplicate.

Figure 1*Conceptual framework*

3.1 Collection of Coconut Milk

45 mL of mature coconut milk was collected from the City of San Jose Del Monte, Bulacan, on the day of the experimentation.

3.2 Collection of Atis Leaves

300 grams of atis leaves were collected from the City of San Jose Del Monte, Bulacan, on the day of the experimentation.

3.3 Collection of Test Organisms

The collection of human head lice followed a modified procedure of the process done in a research study by Bahad et al. (2022). A total of 36 adult head lice were collected on the morning of the experiments to ensure the viability of the test subjects. The lice were collected from one person in the City of San Jose Del Monte, Bulacan, who had not been treated with commercial pediculicides 3 months prior to the study. Head lice were immediately placed in a

glass container with the treated filter paper on the bottom. Exclusively fully intact lice were used, regardless of sex.

3.4 Preparation of Coconut Milk

The coconuts were dehusked and deshelled. Then, the coconut kernels were rinsed, grated, and thereafter pressed to extract the coconut milk. This process was done until 45 mL of coconut milk was collected. Furthermore, it is worth noting that this study uses only the first press of the coconut kernel.

3.5 Preparation of Atis Leaves Extract

The 300 grams of Atis leaves were rinsed with distilled water after collection. They were then crushed in a pestle and mortar with an added 10 mL per 100 grams. The leaves mixed with the solvent were pressed to extract a liquid form and used after 3 days, allowing for more efficient application in the next step.

3.6 Preparation of Test Solutions

The 7-cm-diameter filter paper discs were treated with each solution before being placed at the bottom of their containers. Afterwards, three (3) lice were placed on top.

Setup 1: 7.5 ml of coconut milk and 2.5 ml of Atis leaf extract were mixed and used for the first solution.

Setup 2: 2.5 ml of coconut milk and 7.5 ml of Atis leaf extract were mixed and used for the second solution.

Setup 3: 5 ml of coconut milk and 5 ml of Atis leaf extract were mixed and used for the third solution.

Setup 4: For the control group, Kwell® was used.

Setup 5: Subjects were observed every minute with stimulation with strands of human hair and then declared dead if unresponsive from direct stimulation, as was done in the study by Bahad et al. (2022).

3.7 Data Analysis

For data analysis, the following were applied to the data gathered from all setups. To determine which ratio had the fastest kill time, which will be labeled Eradequine, the mean

was used. To determine whether or not Eradequine was a viable alternative to Kwell, an independent t-test was used. To determine the correlation between the deaths of the lice and Eradequine, as well as in choosing which hypothesis to accept, Pearson r was used, with the variables being time and the deaths observed.

4. Findings and Discussion

Table 1 reveals that the average time it takes for each ratio (coconut milk to Atis leaves) to reach a 100% mortality rate is as follows:

75:25 has an average of 13 minutes and 40 seconds.

25:75 has an average of 11 minutes and 20 seconds.

50:50 has an average of 18 minutes.

Table 1

Pediculicidal activity observed and the mean in minutes

Treatments	Trials	Number of dead head lice (minutes)						Time of total mortality (minutes)	Mean (Minutes)
		5	10	15	20	25	30		
75:25	1	3	3	3	3	3	3	4	13:40
	2	0	1	2	3	3	3	20	
	3	0	1	3	3	3	3	17	
25:75	1	2	2	3	3	3	3	13	11:20
	2	0	2	3	3	3	3	11	
	3	0	3	3	3	3	3	10	
50:50	1	1	2	3	3	3	3	13	18
	2	0	0	3	3	3	3	14	
	3	0	0	1	2	2	3	27	
Kwell	1	0	3	3	3	3	3	4	9:20
	2	0	0	3	3	3	3	12	
	3	0	0	3	3	3	3	12	

Kwell presents the fastest pediculicidal activity with an average of 9 minutes and 20 seconds. Following after is a ratio of 25:75 coconut milk and Atis leaves with an average of 11 minutes and 20 seconds. Thirdly, a ratio of 75:25 coconut milk and Atis leaves with an average of 13 minutes and 40 seconds. Lastly a ratio of 50:50 with an average of 18 minutes. Furthermore, Kwell's commercial packaging instructs users to leave it on the head for 10 minutes. From the data gathered, a ratio of 25:75 coconut milk and Atis leaves has the fastest killing time. These results lead us to reject the null hypothesis (H01), and accept the first alternative hypothesis (Ha1). Therefore, Eradequine will refer to a ratio of 25:75 coconut milk and Atis leaves.

Table 2*t test results comparing 25:75 coconut milk to Atis leaf ratio and Kwell*

Std err	2.81
t-stat	0.71
Df	2.43
p-value	0.54
t-crit	3.65
Lower	-12.24
Upper	8.24
Sig	no

Results between Kwell (M=9.33, SD=4.62) and 25:75 ratio of coconut milk and Atis leaves (M=11.33, SD=1.53) indicate a non-significant difference between Eradequine (25:75 ratio of coconut milk and atis leaves) and Kwell.

To be able to say for certain how Eradequine compares to it regarding the time it takes to kill all the lice, an independent t test with unequal variance was used. This can be seen in table 2, which reports a non-significant difference between the experimental groups and the positive control group. Since there is no significant difference between any of the ratios to the positive control group, a known drugstore product against lice, the results imply to reject the null hypothesis (H02), and accept the alternative hypothesis (Ha4) that Eradequine is a viable alternative to commercial pediculicides.

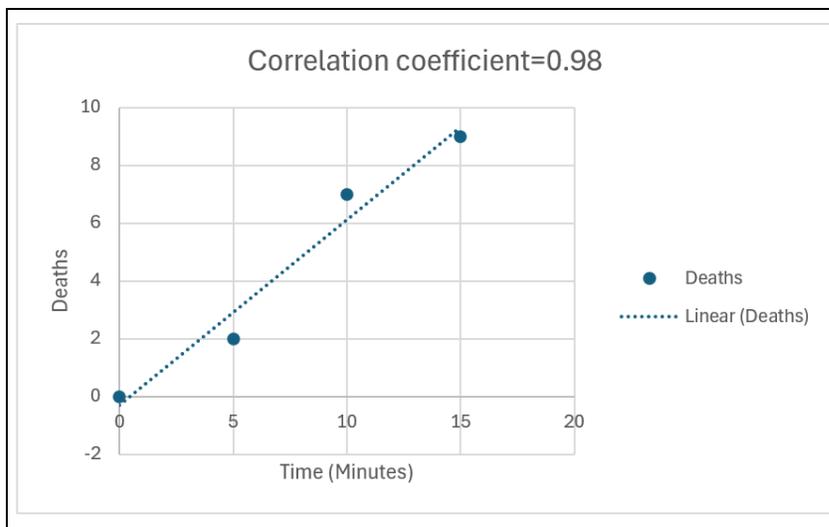
Figure 2*Pearson correlation coefficient testing using time and death of the lice*

Figure 2 reports a highly positive correlation between Eradequine and the death of the lice, $r(2) = .98$, $p = .017$. This means that there is a positive correlation between the experimental treatment and the death of the lice; an increase in the time the lice were treated means an increase in the number of dead lice. Using this, reject the null hypothesis (H_0) and accept the alternative hypothesis (H_a) that there is a positive relationship between Eradequine and the mortality rate of lice. The pediculicidal activity of Atis leaves may be attributed to an insecticidal property inside the leaves named anthraquinones (Nhung & Qoc, 2024). Anthraquinones and its derivatives have been widely used as a non-lethal way for agricultural protection and pest management (DeLiberto & Werner, 2016).

5. Conclusion

This research aimed to create an alternative pediculicide using macerated Atis leaves and coconut milk to combat the prevalence of pediculicide, growing permethrin resistance, harm brought on by pharmaceutical wastes, as well as to utilize and further add to knowledge on the medical properties of coconut and Atis leaves, which may be under-utilized in this aspect.

Based on the findings, all ratios had a 100% mortality rate, with a total of 9 lice killed per group. A 25:75 ratio of coconut milk and Atis leaves achieved total mortality the fastest with an average of 11 minutes and 20 seconds, followed by 75:25 and 50:50. Therefore, Eradequine is a viable alternative to commercial pediculicides. Research further shows that there is a positive correlation between Eradequine and the deaths of the subjects. However, this research limits itself as an in-vitro experiment against adult head lice. Therefore, further studies are recommended. In vivo application of the treatment on actual humans with pediculicide should be done to see how Eradequine would work against lice in their natural environment. Application of the treatment to lice eggs should be used to see how the treatment may affect their hatchability and subsequently prevent the next wave of pediculosis. Similarly, a study on how Atis leaves and coconut milk would affect persons with allergies is highly recommended.

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Institutional Review Board Statement

This study was conducted in accordance with the ethical guidelines set by City of San Jose Del Monte National Science High School. The conduct of this study has been approved and given relative clearance(s) by City of San Jose Del Monte National Science High School.

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