World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(Research Article)

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Total aerobic plate count and onion (*Allium cepa*) root tip assay using banana (*Musa acuminata x balbisiana*) Pseudostem accumulated water with thin-layer chromatography as confirmatory test

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World Journal of Advanced Research and Reviews, 2024, 22(02), 2200–2210

Publication history: Received on 16 April 2024 revised on 23 May 2024; accepted on 25 May 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.22.2.1588

Abstract

Cancer is a group of diseases characterized by the uncontrolled growth of abnormal cells. It can originate from any of the trillions of cells in the human body. Normally, cells grow and divide as needed, but cancer disrupts this process, causing abnormal cells to multiply uncontrollably. According to Monda et al. (2021), the pseudostem of the banana tree possess anticancer activities due to the phytochemicals present, which is why the researchers want to determine whether the accumulated water inside the banana pseudostem also holds the potential to become an alternative anti-cancer treatment. The researchers utilized total aerobic plate counting and *Allium cepa* root tip assay for the researchers to evaluate the potability of the banana pseudostem accumulated water and assess its inhibitory properties for cell growth, respectively. Based on the findings of the study, the total number of aerobic plates is too numerous to be counted (TNTC) and from the ANOVA analysis conducted, there was no significant difference among the cell growth inhibition. To verify this result, thin-layer chromatography was used to determine if there are any phytochemicals present within the sample possessing anticancer properties. No phytochemicals with such characteristics were identified. In conclusion, the banana pseudostem accumulated water is not a suitable alternative for anticancer medications. The researchers recommended considering other factors that might affect the quality of the sample. They also suggested subjecting the data to a more in-depth analysis, such as examining the Mitotic Index and chromosomal aberrations, to obtain more specific information.

Keywords: Banana Pseudostem Accumulated Water; *Allium cepa* Root Tip Assay; Thin-Layer Chromatography; Phytochemicals; Anticancer Potential;

1. Introduction

Cancer, a term that evokes fear and uncertainty, is a complex disease group that is characterized by abnormal cell growth. This particular disease can originate almost everywhere from the trillion cells that compose the human body. Under normal circumstances, human cells grow and divide to form new cells as needed. However, when this orderly process is disrupted, abnormal or damaged cells grow and multiply uncontrollably (National Cancer Institute, 2021).

The impact of cancer in the Philippines and globally is significant. This disease claimed almost 10 million lives globally in 2020, making it one of the top causes of mortality (World Health Organization, 2022). In the Philippines it was reported that the disease killed more than 39,710 people in 2023, accounting for 10.2 percent of the country's deaths. The disease has various risk factors, such as smoking, drinking, infections, and lack of exercise (Statista, 2023).

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Furthermore, numerous efforts have been undertaken to understand the disease better and develop effective treatments. For instance, the National Cancer Institute (NCI) and the American Cancer Society have been at the forefront of cancer research with regards to this matter. Governments worldwide, including the Philippines, have launched various initiatives and programs for cancer prevention and control. In the Philippines, the Disease Prevention and Control Bureau (DPCB) set up the National Integrated Cancer Control Program through the National Integrated Cancer Control Act (NICCA) that aims to decrease the incidence of preventable cancer, prevent and manage its recurrence, and provide access to quality healthcare for its treatment (Philippine Institute for Development Studies, 2023). These efforts include promoting healthier lifestyles, implementing cancer screening programs, and improving access to cancer treatments. Additionally, the Cancer Assistance Fund was established to provide support for patients living with cancer and for cancer survivors (Department of Health, 2022).

Despite these current efforts to remedy the disease, there is still no universal "cure" for it, continuing to ravage the lives of millions in the world. While alternative treatments exist in fighting this disease, such as chemotherapy, surgery, and radiation therapy. They also have their own disadvantages. Chemotherapy is deficient in specificity and has risks of cytotoxicity, induction of multi-drug resistance, and stem-like cell growth (Cheng et al., 2021). Radiation therapy, on the other hand, causes tissue damage, which also includes the destruction of healthy tissues (Marcolongo et al., 2020). Consecutively, surgery also adversely affects the body during and after the procedure. Blood loss and damage to organs in the body are some problems that can arise during surgery while infections, blood clots, and pneumonia can sprout in post-surgery (Stanford Medicine, 2024). Given the turmoil and suffering that cancer has caused the people of the world and the disadvantages of alternative treatments for it, there is a critical need for more anticancer medications with less unfavorable consequences to combat this pervasive disease.

Musa acuminata × balbisiana or more commonly known as "Saba banana" is a popular fruit grown in Southeast Asia (Reginio et al., 2020). It is one of the four main cultivars of bananas in the Philippines that is rich in vitamins and minerals (Moso, Puda, Timaan, & Jolito, 2021). The plant's high nutritional value has led to increasing demand in both local and global markets (Ohagan et al., 2023). In fact, this particular variety accounts for 28% of the total banana production in the Philippines, significantly contributing to both the export market and local consumption (Department of Agriculture, 2021).

The pseudostem of the banana is the part of the plant that resembles a trunk but is actually an aggregate of a central core wrapped with up to 25 leaf sheaths (Subagyo & Chafidz, 2018). It is common that after harvest, this part of the banana is often left in the field or discarded as biowaste as it cannot be used after one harvest (Ramdhonee & Jeetah, 2017). This poses additional problems to the environment if left unmanaged such as contributing to the production of greenhouse gasses when they are burned or become wet (Subagyo & Chafidz, 2018). Contrary to this, pseudostems can still be utilized for other purposes, as they are not just the plant's fruit with a purpose. On the other hand, other parts of the plant can still be utilized for a specific purpose (Subagyo & Chafidz, 2018). The pseudostem has been used as processed feed for hogs, cattle, and poultry (Pillai et al., 2024). With regards to its pharmaceutical potential, studies have shown that the pseudostem possesses disease-preventing effects and anticancer activities due to the presence of various compounds such as phenolics, carotenoids, biogenic amines, phytosterols, and volatile oils (Mondal et al., 2021).

Much like all plants, the saba banana plant relies on capillary action through the xylem to move water from the roots to the leaves and then to the top of the plant for photosynthesis. When the pseudostem is cut and a hole is carved, water begins to accumulate as the exposed fibers and channels attract water molecules, in which surface tension causes the water to creep along the fibers and into the stem (Clare, 2020).

Nutrient transport is responsible for alterations in the water present in the plant (Wegner, 2014). Through the pseudostem, a plant is able to transport nutrients from the soil up to the fruits (Pillai et al., 2024). All plants, encompassing fruits, vegetables, legumes, and grains, generate phytochemicals as part of their immune system, defending against viruses, bacteria, fungi, and parasites (Surampudi, 2024). Phytochemicals are biologically active chemical compounds naturally occurring in plants. These compounds not only protect the plant from threats such as germs, fungi, and bugs, but also play a crucial role in the plant's defense against pathogens and herbivores (Iyer et al., 2023; Soliman et al., 2018). Phytochemicals offer health benefits to humans by serving as medicinal ingredients and nutrients (Koche et al., 2016). They have been used for various purposes such as pharmaceuticals, agrochemicals, flavors, fragrances, coloring agents, biopesticides, and food additives (Oz, A. T., 2017). They also have biological activities such as antioxidant activity, antimicrobial effect, and immune system stimulation. (Wani et al., 2022) Some phytochemicals have been used in traditional medicine. For example, salicin, having anti-inflammatory and pain-relieving properties, was originally extracted from the bark of the white willow tree, and later synthetically produced to become the common, over-the-counter drug aspirin (Science Learning Hub, 2018).

Phytochemicals have shown promising anti-cancer efficacy with their distinct advantages of high efficiency and low toxicity (Royal Society of Chemistry, 2022). They can block initiation or reverse the promotion stage of multi-step carcinogenesis. They can also halt the progression of precancerous cells into malignant ones. Plant-derived compounds have historically led to some of our most useful cancer drugs like paclitaxel, vincristine, etc. (Arif et al., 2022). Further evidence suggests that phytochemicals modulate autophagy and apoptosis, the two crucial cellular pathways involved in the underlying pathobiology of cancer development and regulation. Pharmacological targeting of autophagy and apoptosis signaling using phytochemicals therefore offers a promising strategy that is complementary to conventional cancer chemotherapy (Rahman et al., 2021). While the potential of phytochemicals in cancer treatment is promising, it is through advanced analytical techniques such as Thin Layer Chromatography and *Allium cepa* Root Tip assay that the researchers can truly understand and harness their capabilities.

In relation to this, genotoxicity refers to the ability of chemical agents to damage the genetic material within a cell, thereby causing mutations (Redenna et al., 2017). In the context of anticancer efforts, it was used on TRIM37 on cancer cells and found that through blocking genotoxicity, cancer cells become more vulnerable to drugs that cause DNA damage. This knowledge demonstrates how genotoxicity can be used to identify potential anticancer properties of certain substances (Wu et al., 2018). Given the role of genotoxicity in anticancer research, it is crucial to have effective methods for its assessment. One such method is the *Allium cepa* Root Tip Assay.

Allium cepa Root Tip Assay is a test for chemical screening of potential genotoxicity and cytotoxicity of several types of physical, chemical, and biological agents using the *Allium cepa* plant. It is a cost-effective way as the *Allium cepa* plant possesses large chromosomes that allow them to be easily observed under a microscope to better illustrate certain effects (Bonciu et al., 2018). In terms of anticancer efforts, *Allium cepa* Root Tip Assay has been used to evaluate the cytotoxicity of anticancer drugs (Das et al., 2021).

Phytochemicals and *Allium cepa* Root Tip Assay are all valuable assets in the field of anticancer research that led to the discovery of significant insights regarding the disease. Building on these principles, this study aims to explore a novel approach to cancer treatment. Given the global and local impact of cancer, particularly in the Philippines, and the shortcomings of existing treatments like chemotherapy, surgery, and radiation therapy, it is imperative to develop more potent anticancer drugs with reduced side effects. This study also aims to leverage on the potential of water accumulated in the pseudostems of the Musa acuminata x balbisiana that is native to the Philippines. This study represents an innovative step towards combating this pervasive disease, potentially providing a more targeted and less harmful alternative to existing treatments.

1.1. Statement of the Problem

This study entitled "Total Aerobic Plate Count and *Allium cepa* Root Tip Assay using Banana (Musa acuminata x balbisiana) Pseudostem Accumulated Water with Thin-Layer Chromatography as Confirmatory Test" aims to uncover the types of the microbes present, its anticancer characteristics using its *Allium cepa* root tip assay and the phytochemicals present in Musa acuminata x balbisiana accumulated water.

Specifically, this study will be guided by the following questions:

- How may the potability of banana pseudostem accumulated water be described?
- How may the root growth of *Allium cepa* be affected using different treatments (25%, 50%, 75%, 100%, 300 mM Hydrogen Peroxide, and sterile distilled water)?
- Is there a significant difference in the root growth of *Allium cepa* using different treatments (25%, 50%, 75%, 100%, 300 mM Hydrogen Peroxide, and sterile distilled water)?
- What phytochemical substances are present in banana water as confirmatory chemicals that can be a potential alternative for anticancer treatment?

1.2. Hypothesis

The following hypothesis was tested in the study:

- There is no significant difference in the root growth of *Allium cepa* using different treatments (25%, 50%, 75%, 100%, 300 mM Hydrogen Peroxide, and sterile distilled water).

1.3. Significance of the Study

The aim of this study is to assess the potential anticancer properties of banana pseudostem accumulated water. Given the widespread impact of cancer, affecting millions globally, the pursuit of natural alternatives for its treatment holds promising benefits, as it could aid individuals afflicted with the disease to have a secure option with fewer associated side effects. Through this study, the medical community in the Philippines has the potential to develop anticancer medications that are not only of high quality but also affordable and safe. Hence, the findings of this study will benefit the following:

Community. This study will benefit people living in provinces and far from cities, where high-quality medicines might be less accessible. Through this study, they will gain awareness about the importance of natural remedies readily available within their community.

Pharmaceutical Scientists. To serve as the basis for future experiments and the formulation of new alternative medicines. Through this study, scientists can improve the qualities of drugs and even formulate medicines from banana water.

Medical Fields. To assist in the formulation of medicines using natural components, this study can contribute to the development of safer and more affordable alternatives.

Department of Health. With the guidance of this research, the Department of Health can use this study as the basis for their future medicinal purposes.

Future Researchers. The importance of this study lies in its potential to serve as a foundational resource for future researchers, providing valuable insights into the importance of banana water and its potential anti-cancer properties.

1.4. Scope and Limitation of the Study

This study tackles the potability of the banana pseudostem accumulated water through total aerobic plate count, in which they identified the number of microbes present in the sample.

This study also covered the ability of Musa acuminata × balbisiana accumulated water to inhibit the cell division of *Allium cepa* roots using *Allium cepa* root tip assay, to uncover its inhibitory characteristics on cell growth. The researchers also utilized thin-layer chromatography to determine the phytochemicals present in the sample.

This study was conducted during the school year 2023-2024. The pseudostem accumulated water was collected and prepared at Mandili, Candaba, Pampanga whilst the collection, preparation and the testing for the *Allium cepa* root tip assay was done at Tibagan, San Miguel, Bulacan. The researchers have dedicated 18 days for the whole testing procedure and a day for the data analysis and conclusion formulation. The phytochemical analysis through thin-layer chromatography was conducted at Central Luzon State University and the total aerobic plate count was done at Adamson University.

1.5. `Definition of Terms

The following operational definitions of the terms used in this study are provided to aid comprehension.

- Anti-cancer. The researchers aim in developing this research. It is the property tested to see if the banana pseudostem accumulated water is a potential alternative solution for cancer.
- *Allium cepa* Root Tip Assay. The test that was utilized to evaluate the cytotoxicity of banana pseudostem accumulated water.
- Banana Pseudostem Accumulated Water. The material used for finding a potential anti-cancer. It was collected from a Musa acuminata x balbisiana tree by cutting it carefully and carving a hole in its stem, allowing the accumulation of banana water.
- Hydrogen Peroxide. The positive control used in the study. It is the substance that acted as the ideal reference for the genotoxicity of banana pseudostem accumulated water.
- Phytochemical. The compounds found in the banana water after going through phytochemical analysis.
- Thin-Layer Chromatography. The technique selected by the researchers for identifying the phytochemicals present within the banana pseudostem accumulated water.
- Total Aerobic Plate Count. The quantity used to indicate whether microorganisms are present in the banana pseudostem accumulated water or not.

2. Methodology

The information about the procedures followed by the researchers during the experimental process are shown here. The statistical tool that was utilized for data analysis was also provided.

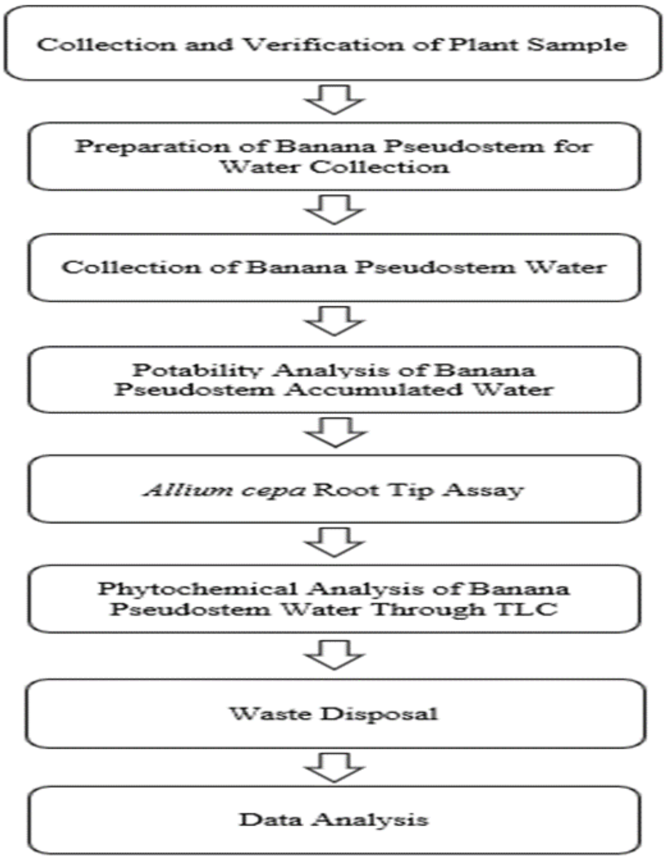

Figure 1 Flow Chart of Activities

2.1. Collection and Verification of Plant Sample

The banana tree samples were taken from Brgy. Mandili, Candaba, Pampanga and were later on identified and certified by Central Luzon State University (CLSU) as a species of Musa acuminata × balbisiana (ABB Group) 'Saba'.

2.2. Preparation of Banana Pseudostem for Water Collection

The researchers constructed a makeshift isolated area using wood and plastic cover to prevent outside contamination. The banana tree was then carefully cut down using a large knife at the height of the waist and a hole was carved within the remaining portion of the pseudostem, enabling the gradual accumulation of banana water.

2.3. Collection of Banana Pseudostem Accumulated Water

After allowing the water inside the pseudostem to accumulate for one day, a sterile syringe was utilized to collect it. Two liters of the sample were collected and transferred to a sterile container that was stored for further use.

2.4. Potability Analysis of Banana Pseudostem Accumulated Water

A 500 mL banana pseudostem accumulated water was brought to Adamson University, where they conducted a potability analysis. They performed microscopy on the sample and determined the quantity of colonies (measured in Cfu) that is present in the sample.

2.5. *Allium cepa* Root Tip Assay

Fifty healthy bulbs of onions were sourced from the local market. Once selected, the researchers carefully peeled the outer layers of these onions. After peeling, the onions were placed in a container filled with distilled water, allowing the onions to germinate for a period of 3 days (Cabuga Jr. et al., 2017). Eighteen onions that have germinated successfully were chosen by the researchers and subjected to different set-ups.

Following the germination period, a positive control was introduced to the experiment, 300 mM of Hydrogen Peroxide was utilized. Alongside the positive control, distilled water is used as the negative control. (Akwu et al., 2019) Finally, the application of different concentrations of banana water (25%, 50%, 75%, 100%) was implemented by the researchers as their experimental group. Three replicates were done for each treatment.

The roots were immersed in the container for 48 to 72 hours, during which it was made sure that the root tips were fully submerged. After the incubation period, the onion bulbs were carefully removed from the test solutions. Roots are carefully measured through the use of measuring tape and tallied through their respective group (Sheela & Thoppil, 2017).

2.6. Thin-Layer Chromatography of Banana Pseudostem Accumulated Water

The researcher brought one liter of the sample to the Central Luzon State University to undergo analysis through Thin-Layer Chromatography (TLC). In there, a TLC plate spotted with the sample was utilized and submerged in a sealed chamber containing a solvent. After the solvent front has reached a specified level, the plate was immediately taken out and allowed to dry before proceeding to the visualization step (Li, 2014). The identified compounds were then sent to the researchers via email.

2.7. Waste Disposal

After conducting the experiments, the first step is to collect the banana pseudostem water and any other waste materials in a suitable container. The container should be clean and have a tight-fitting lid to prevent any spills. The next step is to segregate the waste based on its type. For instance, solid waste should be separated from liquid waste. This segregation makes the disposal process more efficient. Once segregated, the waste should be stored in a safe place until it can be disposed of. The storage area should be cool, dry, and out of reach of children and pets to prevent any accidents. Finally, the onions used in the experiments, whether they are contaminated or not, will be discarded in compliance with the guidelines set by the authorized research institution.

2.8. Data Analysis

The potability of the banana pseudostem accumulated water will be confirmed through microscopy and the total number of aerobic plate counts present in the sample.

The mean root growth of *Allium cepa* using different treatments will be measured and tallied, in which the trend will be described.

Analysis of Variance (ANOVA) will be utilized to determine the significant difference between the influence of different concentrations of banana pseudostem water (25%, 50%, 75% and 100%) and the control substances (300 mM Hydrogen Peroxide and sterile distilled water) in the root growth of *Allium cepa*.

The presence of phytochemicals acting as confirmatory to the anticancer potential of banana pseudostem accumulated water through Thin-Layer Chromatography.

3. Results and discussion

This chapter deals with the presentation, analysis and interpretation of the data collected and the results of the statistical treatment employed in the study with the purpose of evaluating whether the water accumulated in the banana pseudostem possesses potential as an alternative for anticancer medications.

3.1. Potability Analysis

According to the US FDA, the aerobic plate count (APC) is intended to indicate the level of microorganisms in a product and to be considered as to be TNTC (Too Numerous To Count) it should have a count of more than 300 Cfu (Colony

Forming Units). Based on the results of APC, the sample has a microbe count of TNTC up to a dilution factor of 5 (See Appendix C). The results imply that the banana pseudostem accumulated water is not potable, agreeing with the study conducted by Alvarez et al. (2017) showing that a sample having to be found containing numerous microbes is not suitable for direct human consumption.

Table 1 Total Aerobic Plate Count Results from Adamson University

Dilution Factor	**Colony Forming Units (CFU)**
^1	TNTC
^2	TNTC
^3	TNTC
^4	TNTC
^5	TNTC

Note: TNTC stands as Too Numerous To Count

3.2. *Allium cepa* Root Tip Analysis: Trend Analysis and ANOVA

The *Allium cepa* root tip assay is utilized to detect the genotoxic impacts of a substance. A drug with genotoxic properties can induce cell death and inhibit cell growth, indicating its potential as an anticancer treatment (Sharma et al., 2019).

Findings indicate that the highest inhibition of root growth occurs at the positive control with the 100%, 75%, 50%, 25%, and negative control following in sequence. This suggests that different concentration levels affect the results of the investigation. As the concentration of the banana pseudostem accumulated water increases, the inhibition rate also increases, slowing down root growth. The Analysis of Variance (ANOVA) results in Table 3 reveal a calculated p-value of 0.25538, which is higher than the significance level of 0.05, suggesting an insignificant difference in *Allium cepa* root growth among the treatments. While there is a positive trend in concentration leading to an increase in inhibition rate, the ANOVA results suggest that the data lacks significance. The *Allium cepa* root tip assay is commonly used to assess a sample's potential in restricting cell growth by observing its effect on the roots of *Allium cepa*. Although inhibition of root growth typically indicates disruptive effects on cell division, the data from the *Allium cepa* root tip assay regarding banana pseudostem accumulated water does not show statistical significance. Therefore, it cannot be conclusively stated how well the banana pseudostem accumulated water could influence cellular growth, consistent with the findings reported by Cabuga et. al (2017), depicting how the root growth of *Allium cepa* is connected to the sample's ability to affect cell division.

Table 2 Average of Root Growth in *Allium cepa* Assay

Treatments	**A**	**B**	**C**	**D**	**E**	**F**
Root Growth (mm)	6.33^{a}	5^{a}	2.33a	1.66^{a}	1.33^{a}	8.33^{a}
p-value	0.25538					

Note: Different superscripts mean there is a significant difference. The letters pertain to different treatments A (25%), B (50%), C (75%), D (100%), E (Positive Control), F (Negative Control).

3.3. Thin-Layer Chromatography

The results collected from the thin-layer chromatography showed that sugars are the only phytochemical present in banana pseudostem accumulated water, while essential oils, sterols, phenols, fatty acids, anthraquinones, coumarins, anthrones, tannins, flavonoids, steroids, alkaloids, and amino acids are absent. According to Rudzińska et al. (2023), phenols, carotenoids, and alkaloids are phytochemicals exhibiting anticancer potential, which is absent in the sample based on the thin-layer chromatography done in Central Luzon State University confirming the *Allium cepa* Root Tip Assay results.

Table 3 Thin Layer Chromatography (TLC) Results from Central Luzon State University

Phytochemicals Screened	Present/Absent
Sugars	Present
Essential Oils	Absent
Sterols	Absent
Phenols	Absent
Fatty Acids	Absent
Anthraquinones	Absent
Coumarins	Absent
Anthrones	Absent
Tannins	Absent
Flavonoids	Absent
Steroids	Absent
Alkaloids	Absent
Amino Acids	Absent

4. Conclusions

This study focuses on evaluating the potability of banana pseudostem accumulated water and its potential as an anti-cancer substance and a cost-friendly alternative for patients with cancer. Based on the findings of the study, the following conclusions were formulated:

The sample of banana pseudostem accumulated water is determined to be not safe for direct consumption, as the total quantity of aerobic plates found is too many to be counted, indicating that there are many microbes present in the sample.

Based on the collected average root growth from each set-up, the highest rate of inhibition occurs at Treatment E (positive control) with Treatment D (100%), Treatment C (75%), Treatment B (50%), Treatment A (25%) and Treatment F (negative control) following in sequence. From this, it can be inferred that as the concentration of banana pseudostem accumulated water becomes higher in the solution, the greater it inhibits cell division but further testing is required as ANOVA results show that it is not significant.

Based on the Analysis of Variance (ANOVA) utilized on the results gathered from the *Allium cepa* Root Tip Assay, there is no significant difference between the influence of different concentrations of banana pseudostem water (25%, 50%, 75% and 100%) and the control substances (300 mM Hydrogen Peroxide and sterile distilled water) in the root growth of *Allium cepa*.

The results confirmed that the banana pseudostem accumulated water does not have any anti-cancer potential, as there are no phytochemicals found in the sample except for sugars.

Recommendations

Based on the findings of this study, further research is recommended to explore the potential of banana pseudostem water as an anti-cancer agent. In particular:

Future researchers should consider the location of the banana tree where the banana pseudostem accumulated water was collected as a factor affecting the number of microbes present. They should also look for other methodologies that could assist in identifying the specific type of microbe present in the sample. Additionally, the sample should be pasteurized because the potability analysis reveals that there are too numerous microbes present in the banana pseudostem accumulated water.

Larger sample sizes could be utilized by the researchers to further make the trend illustrated in the results more accurate.

The grown roots of *Allium cepa* should be subjected to microscopy in order to calculate the mitotic index and check for chromosomal aberrations so that the effect of the banana pseudostem accumulated water on the cell division could be specifically described.

Future researchers could consider collecting banana pseudostem accumulated water from other species of banana and letting it undergo the same confirmatory tests.

Compliance with ethical standards

Acknowledgements

The researchers would like to express their deepest gratitude to the people behind the success of this study. The researchers would like to extend their warmest appreciation to those who expressed and showed their support and lent their priceless time to make this study possible. Those people are namely:

- First, Dr. Rich Milton R. Dulay, a Biology teacher at Central Luzon State University (CLSU), for his assistance in the phytochemical analysis during the visit of the researchers in their school, which significantly contributed to the completion of this research and to Mr. Airon Riel L. Rivera, a microbiologist, for helping the researchers bring the sample to Adamson University and analyze its potability.
- Mr. Marciano V. Cruz, Jr., School Principal IV, Dr. Cherilyn R. Goyena, Assistant Principal II for Academics, and Dr. Joselito G. Milan, Assistant Principal II for Operations and Learners Support, for their moral support and unwavering knowledge in guiding us with this research.
- Also, to Mr. Lenard Francis DP. Parulan and Mr. Richard Louie P. Lamorena for being good companions and a great help to the research.

Disclosure of Conflict of interest

All authors contributed positively to the writing of this manuscript and there no conflict of interest as agreed to the content of this research. The researchers have no affiliations with or involvement in any organizations, or entities with any financial, and non-financial interest in the subject matter, materials, and methods discussed in this study.

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