CYTOTOXIC CAPABILITIES OF KATUDAY (*Sesbania grandiflora Linnaeus*) LEAVES AND SEEDS EXTRACT USING Allium cepa ASSAY

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In partial fulfilment of the
requirements in the subject
Research Project

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This investigatory project entitled, **Cytotoxic Capabilities of Katuday (*Sesbania Grandiflora Linnaeus*) Leaves and Seeds Extract Using Allium Cepa Assay,** prepared and submitted by **Allyssa Mae C. Cantos, Kim Jeyeon A. Dato and Daphne Kate V. Valdez** in partial fulfillment of the requirements for the subject, **Research Project**, has been examined and is recommended for Oral Examination.

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- *The Researchers*

DEDICATION

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- *The Researchers*

RESEARCH ABSTRACT

TITLE:	**CYTOTOXIC CAPABILITIES OF KATUDAY (Sesbania grandiflora Linnaeus) LEAVES AND SEEDS EXTRACT USING Allium cepa ASSAY**
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Key Words: Katuday, Allium cepa assay, Cytotoxic Drug, Cancer, Mitotic Index, Nanomaterials, Cell proliferation, Chemotherapy, Cytogenetic, and Antiproliferative

ABSTRACT: Nowadays, cancer is a major public health problem worldwide. Cancer treatment relies on chemotherapy using cytotoxic drugs and radiation therapy which can be harmful to the health. Research has progressed to investigate the possible qualities and applications of plant extracts for the creation of prospective nanomaterial-based therapeutics for diseases such as cancer. In connection, katuday is abundant in bioactive substances which act as cytotoxic agents. So, the researchers conducted a study entitled Cytotoxic Capabilities of Katuday (Sesbania grandiflora Linnaeus) Seeds and Leaves Extract Using Allium cepa Assay. In testing the cytotoxic capabilities of katuday seeds and leaves, the researchers utilized three (3) treatments: T0 – 100% distilled water, T1 – 100% katuday leaves extract, and T2 – 100% katuday seeds extract. Ethanolic extraction was utilized, and the phytochemical analysis revealed that katuday seeds and leaves contain physiologically active constituents like flavonoids, saponins, tannins, and triterpenoids

which helps in cytotoxic activity. In terms of mitotic index, T2 – katuday seeds extract yielded the best result with a mean of 8.38% which is the lowest mitotic index mean. Moreover, T-test for Independent Samples was also utilized, and the result showed that there is no significant difference between the katuday seeds and leaves extract implying that both parts of the plant are cytotoxic agent. Therefore, this study suggests that researchers can develop a product which contains katuday seeds and leaves which can be used as a cytotoxic drug.

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CHAPTER I

INTRODUCTION

Life revolves around maintaining your health. The quality of health affects every aspect of life (A higher branch, 2020).

In this present time, cancer is a major public health problem worldwide. According to Cancer Research UK (2018), there are 17 million new cases of cancer worldwide. Additionally, according to the University of the Philippines' Institute of Human Genetics, National Institutes of Health (2022), in the Philippines, 189 of every 100,000 Filipinos are affected by cancer.

The number of cancer cases worldwide is predicted to reach 28.4 million in 2040, up 47% from 2020. This increase will be more pronounced in transitioning (64% to 95%) than in transitioned (32% to 56%) countries due to demographic changes, though this may be made worse by rising risk factors brought on by globalization and an expanding economy (Sung, 2020).

On the brighter side, a lot of progress has been made in the treatment and prevention of cancer, and it has been a constant battle everywhere. According to Cancer Research in United Kingdom (2020), cancer is a condition characterized by an uncontrollable or irreversible cell proliferation in the human body. This results in the growth of cancerous tumors that have the potential to spread chemotherapy, radiation, and medications made from chemicals that are currently used in therapies.

According to the Department of Health Republic Act 11215 or the National Integrated Cancer Control Act seeks to implement a national framework to fight cancer. It has been established and aimed at decreasing the incidence and deaths caused by cancer in adults and children. And data from the Department of Health showed that the estimated incidence of cancer was up to eight (8) deaths per day for childhood and up to eleven (11) new cases and seven (7) deaths per hour for the adults. This means over 110,000 new cancer cases and over 66,000 deaths each year.

Cancer treatment relies on chemotherapy using cytotoxic drugs, radiation therapy, and surgery. Today, a variety of cytotoxic drugs have been reported to combat cancer. Most of these drugs are inadequate not only because of their therapeutic efficacy but also because of the undesirable side effects (Pajaniradje et al, 2014).

Cancer Research UK (2020) stated that, chemotherapy is one treatment that can put people under a lot of stress and worsen their health. As a result, emphasis is placed on employing complementary and alternative therapies to combat cancer.

Moreover, cytotoxic drugs also known as antineoplastic drugs or chemotherapy drugs kill or slow down the growth of living cells. They are used to treat cancer and other medical conditions such as rheumatoid arthritis and multiple sclerosis (Auteroa, 2020).

Cytotoxic evaluation uses a number of endpoints, such as cell viability, cell cycle function and control, cell membrane integrity, deoxyribonucleic acid synthesis, metabolic side effects, and apoptosis, to help figure out how damaged and alive cells are. There are numerous techniques for determining cytotoxicity. Regarding its specificity and sensitivity, each approach has its own set of advantages and disadvantages. Consequently, a suitable

bioassay should be chosen based on the investigation and the intended outcome (Soloneski & Larramendy, 2021).

Allium Test is often employed to assess cytotoxic and genotoxic effects, including chromosomal aberrations, micronucleus, and mitotic cycle disruption. The use of the Allium Test to evaluate the effects of soil and water samples from radiation-contaminated areas was also beneficial. In cases of mixed contamination of environmental compartments with radionuclides and chemical compounds, the Allium Test was suggested as a useful method for genotoxicity assessment (Oudalova et al., 2017).

In connection to the study above, plants are reported to have a long history in the treatment of cancer. The use of plants and plant-based products for cancer treatment is rapidly growing in medical practices (Pajaniradje et al, 2014). Overall, Allium Test has been shown to be a very practical, highly sensitive, and educational cytogenetic method for quick screening of radionuclide and ionizing radiation pollution.

Numerous plant species are currently utilized to treat or prevent cancer. Multiple studies have identified plant species having anticancer qualities, with particular emphasis on those utilized in traditional herbal treatment in developing nations (Ochwang'I, 2014).

Polyphenols, brassinosteroids, and taxols have been found and isolated from terrestrial plants for their anticancer effects (Greenwell M, 2015).

On the other hand, according to Pajaniradje et al, (2014), the alcoholic extract of *S. grandiflora* had antiproliferative effects on lung cancer cells in particular. *S. grandiflora* causes cancer cells to die primarily through apoptosis. A possible G/S arrest, as confirmed by decreased cyclin depression, may have caused apoptosis. In lung cancer cells, the

mechanism of action of the methanolic extract may involve a route that inhibits Neurofeedback activity. The findings of this investigation have led to the discovery of a novel plant source with powerful antiproliferative and apoptotic effects.

S. grandiflora is abundant in a variety of bioactive substances, and it frequently produces significant amounts of bioactive metabolites such flavonoids, steroids, alkaloids, saponins, and phenols (Deepthi, et. al., 2022).

The study was conducted at Don Mariano Marcos Memorial State University – North La Union Campus laboratory and was conducted all throughout the first and second semester of the academic year August 2022 – June 2023. This study focused on comparing the cytotoxic capabilities of katuday ***(****Sesbania grandiflora Linnaeus****)*** leaves and seeds using *Allium cepa* assay. It was delimited to two (2) treatments. Leaves and seeds of katuday **(***Sesbania grandiflora Linnaeus***)** were used and no other part of the **(***Sesbania grandiflora Linnaeus***)** tree will be utilized.

The findings of this investigation contributed a lot to the field of medicine. The results of this study surely contributed pertinent information to those who are not yet familiar with the plant itself and also determined which part of the plant is the most effective in killing living cells. This study is a useful initial step in determining the potential toxicity of a test. The bioactive components of the plant were also determined that helped in determining the health benefits of the said plant. This study also helped the different Cancer Organizations that conduct research about cancer. Furthermore, the findings of this study provide scientific support for the use of katuday **(***Sesbania grandiflora Linnaeus***)** in nutrition.

This study aimed to determine the cytotoxic capabilities of katuday **(***Sesbania grandiflora Linnaeus***)** leaves and seeds using *Allium cepa* assay. Specifically, it sought to answer the following questions:

1. What bio-active components of katuday **(***Sesbania grandiflora Linnaeus***)** leaves and seeds extract could help in reducing the production of cancer cells?
2. What is the mitotic index of each treatment?
    a) T_0 – control group
    b) T_1 – 100% katuday leaves extract; and
    c) T_2 – 100% katuday seeds extract
3. Is there a significant difference among the treatments in terms of mitotic index?
4. Which among the treatments yielded the highest result?
    b) T_0 – control group
    b) T_1 – 100% katuday leaves extract; and
    c) T_2 – 100% katuday seeds extract

To further discuss the rudiments of this study, the following terms were defined conceptually to ensure clarity and better understanding.

Cancer - It refers to a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body.

Cytotoxic drug - A drug that slow down the growth of living cells and used to prevent and treat cancer.

Cell proliferation - a rise in cell population brought on by cell division and growth.

Chemotherapy - the use of chemicals to treat disease, particularly the use of cytotoxic medicines and other treatments to treat cancer.

Nanomaterials - drugs that can target particular organs or cells in the body, including cancer cells.

Cytogenetic - examining cells to check for chromosome abnormalities, such as missing, additional, damaged, or rearranged chromosomes.

Antiproliferative - help prevent the spread of cancerous cells into nearby tissue.

Allium Cepa Assay – It is used to assess cytotoxic and genotoxic endpoints such as chromosomal aberrations, nuclear alterations, root growth inhibition, and mitotic index alterations.

Katuday - It is an efficient supply of vitamin a, folate, thiamin, niacin, and vitamin c. flowers additionally render ample quantities of magnesium, phosphorus, potassium, and selenium. It also contains saponins and sesbanimide which possess sturdy antibacterial and antimicrobial properties and detoxify the system.

Mitotic Index – It the measure percentage of cells undergoing mitosis in a given population of cells.

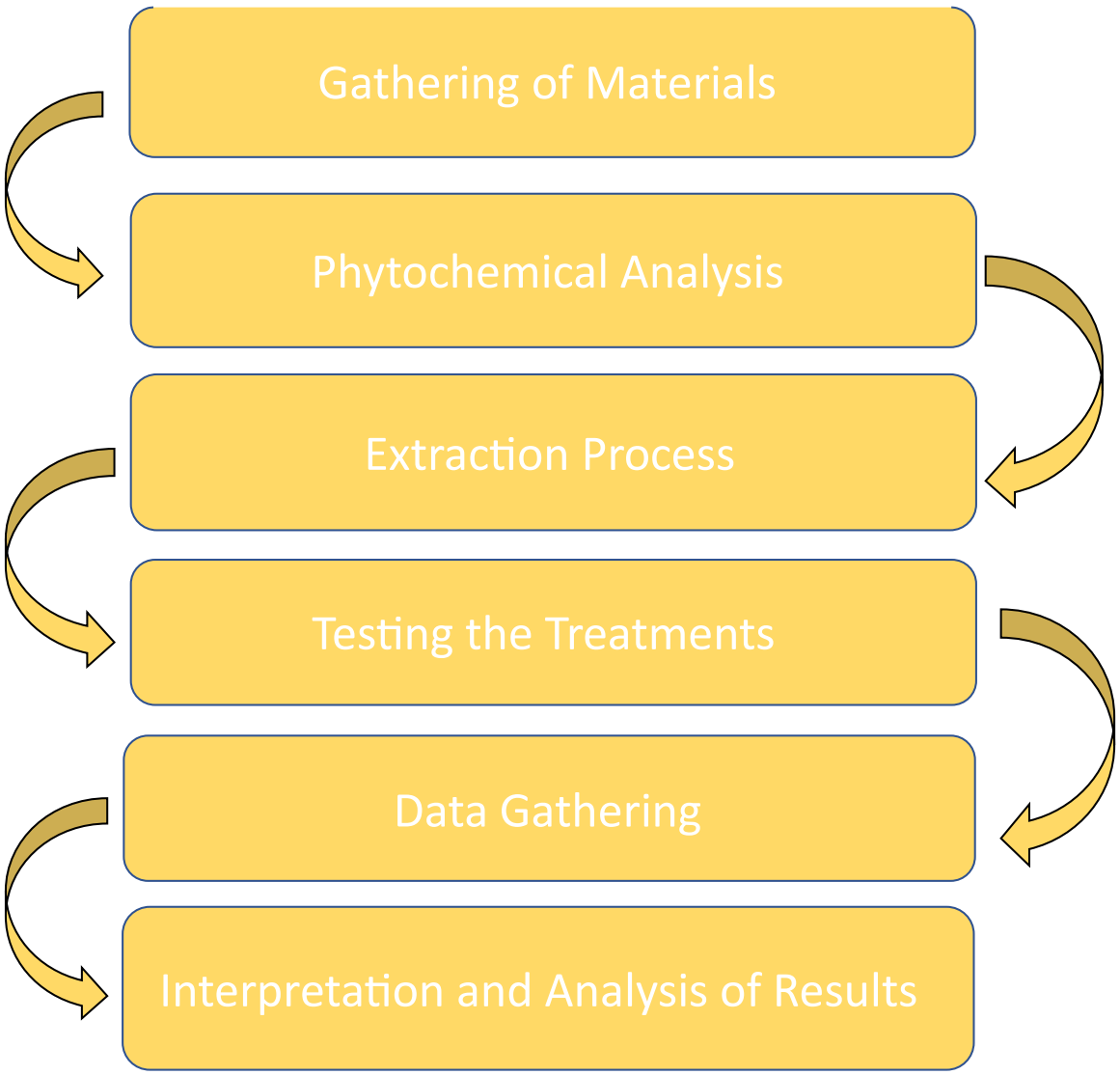

Figure 1. The Flow of Data Gathering Process

CHAPTER II

METHODOLOGY

This chapter focused on the procedures that were done by the researchers. The research design, materials, equipment, general procedures, data collection method, data processing, and ethical considerations were then included.

Research Design

This study used Quantitative Experimental Design as in determining the cytotoxic capability of katuday ***(****Sesbania grandiflora Linnaeus****)*** leaves and seeds extract using *Allium cepa* assay. The study was conducted with a scientific process where a set of variables were kept constant while the other were measured as the subject of experimentation (Bhat, 2019).

In particular, a post-test only control group design was used, in which test units were randomly assigned to the experimental and control groups. The experimental group was exposed to treatment, and both groups were measured subsequently (Insights Association, 2017). This specific design allows for the comparison of two groups while removing the possibility of confounding variables. It is the most precise type of experimental design since it uses statistical analysis to support or refute a hypothesis. It is the only kind of experimental design that allows the researcher to vary the variables and create a cause-and-effect link within a group (Bhat, 2019).

The length of the onion's roots was kept constant while the type of extract was manipulated and served as the independent variable of the study. The cytotoxic potential of

katuday ***(****Sesbania grandiflora Linnaeus****)*** leaves and seeds extract were assessed utilizing this specific design. This design was used to accept or reject the hypothesis presented.

Materials and Experementation

The katuday ***(****Sesbania grandiflora Linnaeus****)*** leaves and seeds were gathered in Santol, La Union. On the other hand, the onions were bought in Bangar, La Union. The equipment such as beakers, blade, scalpel, droppers, digital projection microscope, glass slides, cover slips and chemical solutions were obtained from the laboratory of the Don Mariano Marcos Memorial State University – North La Union Campus. Proper protocols were followed in handling the equipment and chemicals.

General Procedures

a) Gathering of Materials

The katuday ***(****Sesbania grandiflora Linnaeus****)*** leaves and seeds were gathered in Santol La Union. The seed sample was air dried and placed in a (2) two medium size ziplock and labelled properly for ethanolic extraction at Lorma Colleges, Carlatan, City of Fernando. As for the onions needed for making the *Allium cepa* assay were gathered in Bangar, La Union.

b) Phytochemical Analysis

The phytochemical analysis and screening of katuday ***(****Sesbania grandiflora Linnaeus****)*** leaves and seeds was based on the study of Patil, et al., 2022. The researchers reviewed this study to verify if the katuday ***(****Sesbania grandiflora Linnaeus****)*** leaves and seeds have physiologically active constituents like flavonoids, saponins, tannins, and triterpenoids which helps in cytotoxic activity. *S. grandiflora* is abundant in a variety

of bioactive substances, and it frequently produces significant amounts of bioactive metabolites such flavonoids, steroids, alkaloids, saponins, and phenols (Deepthi, et. al., 2022).

c) Extraction of Plant Samples

Katuday ***(****Sesbania grandiflora Linnaeus****)*** leaves and seeds were submitted at the College of Pharmacy, Lorma Colleges, City of San Fernando, La Union for plant ethanol extraction following the Soxhlet method. According to Quy Diem Do, et. al., (2014), ethanol is a reliable solvent for polyphenol extraction and is safe to consume.

d) Treatments and Preparation

Nine (9) onion bulbs were procured by the researchers for the preparation of *Allium cepa* assay. One (1) kilogram of katuday ***(****Sesbania grandiflora Linnaeus****)*** leaves and seeds were gathered and washed with tap water and air-dried for twenty-four (24) hours at room temperature.

According to Brenner (2018), distilled water is used in science experiments to ensure a fair test result. Distilled water does not really have anything in it, and since its inert, tests done for science projects won't be affected by it. When carrying out several science experiments or projects, using pure water as a control element will not affect the outcomes of the tests. In the event if there were minerals or living things present in the water, this might result in skewed, unreliable results.

The ethanol extract of katuday ***(****Sesbania grandiflora Linnaeus****)*** leaves and seeds were measured using a test tube in accordance with the different treatments: T0 which was the 100% distilled water, T1, was the 100% extract of katuday leaves and

T2, was the 100% extract of katuday seeds. The measured extracts were placed in nine (9) identical test tubes.

e) Allium ceoa Assay Preparation

Two (2) kilograms of onions were procured at Bangar, La Union. Before the experiment began, onion bulbs were kept at room temperature for two (2) weeks. After washing the bottom portion of the onion bulb and removing the upper portion of the bulb and the shortened stem, on top of the 100 mL beaker filled with regular water. The standard protocol employed is according to the study of Guterriez (2016), and the roots were treated with three (3) distinct treatments when they reached a length of 1.5–2 cm (2016). T0, 100% distilled water, T1, 100% extract of katuday leaves and T2, 100% extract of katuday seeds for twenty-four (24) hours. The treatments were replicated three (3) times.

f) Allium cepa Assay

The grown roots of onion bulbs were cut into two (2) centimeters and submerged in T0, 100% distilled water, T1, 100% extract of katuday leaves and T2, 100% extract of katuday seeds for twenty-four (24) hours. After that, each root tips were then removed on the containers and were placed on clean glass slides. The very tip of the roots were separated using a blade and treated with one (1) drop of Hydrochloric acid which was used to separate the cells by breaking down the tissue which binds cells together. Two (2) to three (3) drops of Acetocarmine was then used to stain the root tips so that the coiled chromosomes during different stages of mitosis present in the onion root tip cells can be visualized perfectly then the root tips were left

for fifteen (15) minutes. Afterwards, the slide was heated using alcohol lamp and the excess stain was removed. The slide was then covered and fixed using a nail polish. Lastly, the slide was observed in a digital projection microscope (Cx114lcd) with 40x magnification to view the different processes under mitosis to determine the mitotic index of each treatment. According to NC Community Colleges (2020), focusing the microscope with 40x objective would result to a close enough view of the chromosomes in each phase of the mitosis.

g) Data Gathering Process

The cytotoxic capability of the katuday ***(****Sesbania grandiflora Linnaeus****)*** leaves and seeds ethanolic extract using *Allium cepa* assay was evaluated based on mitotic index. Each 1.0 mL concentration of katuday ***(****Sesbania grandiflora Linnaeus****)*** extract included the seeds and leaves. The desired quantity of *Allium cepa* roots were exposed to the extract of katuday ***(****Sesbania grandiflora Linnaeus****)*** seeds and leaves. After 24 hours, the roots of *Allium cepa* were examined, and the number of dividing cells were counted to determine the mitotic index. Using this formula, the quantity of chromosomal abnormalities and the number of metaphase cells scored were determined. The effectiveness of the plant extract increases as the rate of mitotic index lowers.

Data Management

For data analysis, the treatments were made available for additional testing to determine the cytotoxic potential of katuday ***(****Sesbania grandiflora Linnaeus****)*** leaves and seeds extract using the *Allium cepa assay*, in an effort to identify a plant with therapeutic

potential for treating cancer. The study followed a structured process to examine the cytotoxicity of the plant sample considering the aforementioned issues. The chemical elements on katuday ***(****Sesbania grandiflora Linnaeus****)*** seeds and leaves came first. The extract of the plant sample went through preliminary testing, specifically phytochemical analysis to identify the active components present on the plant sample, and the results were used to gauge the cytotoxic potential of katuday ***(****Sesbania grandiflora Linnaeus****)*** seeds and leaves. The cytotoxic potential of katuday ***(****Sesbania grandiflora Linnaeus****)*** seeds and leaves are next in terms of mitotic index. After twenty-four (24) hours of treatment and exposure to various doses of the plant extract sample, the roots of the *Allium cepa* were counted under a digital projection microscope (Cx114lcd) to determine the mitotic index of the proliferating cells.

The mitotic index was calculated using the formula:

$$\text{Mitotic index (MI)} = \frac{Number\ of\ dividing\ cells}{Total\ number\ of\ cells\ examined} \times 100$$

The lowered mitotic index shows that cell division is occurring at a slower rate, suggesting that the plant extract is cytotoxic. The findings established the degree of efficacy of each therapy, which is related to the study's second goal, which is to identify which treatment produces the greatest outcomes when using various concentrations of plant extracts. The best results came from the therapies that produced the desired results after experimentation. Lastly, all the data being collected were encoded in Excel and using Ttest for Independent Samples as the statistical tool with an alpha value of 0.01 as the margin of

error, the significant difference between the treatments were calculated. Following the procedure and conclusion, the results were interpreted as follows.

Throughout the study, the researchers exemplified honesty, fairness, and integrity. The findings of this experiment were reported accurately and without bias. Proper protocols were followed in conducting the experimentation. All the processes that were conducted were based on past studies connected to the topic. Studies and other works of authors that are presented as related studies were be cited properly using American Psychological Association or APA referencing to avoid plagiarism.

CHAPTER III

RESULTS AND DISCUSSION

This chapter presents the results and findings on the study. Results of the phytochemical analysis, mitotic index, and statistical analysis were presented on this part with corresponding interpretations.

Table 1. Phytochemical Analysis of Katuday *(Sesbania grandiflora Linnaeus) Seeds and Leaves*

Sr. No.	Phytochemical constituents	Leaves	Seeds
1.	Alkaloids	+	+
2.	AA / Proteins	-	+
3.	Carbohydrates	+	+
4.	Coumarin	-	-
5.	Flavonoids	-	+
6.	Glycosides	-	+
7.	Phytosterol	-	-
8.	Phenols	-	-
9.	Saponin	-	+
10.	Steroids	+	+
11.	Tannins	+	+
12.	Triterpenoids	+	-

*Legend: (-) negative; (+) positive.

The phytochemical analysis of the katuday ***(Sesbania grandiflora Linnaeus)*** seeds and leaves extract as shown on table 1. According to Patil, et. al. (2022), katuday leaves extract contains significant amounts of alkaloids, carbohydrates, steroids, tannins, triterpenoids. However, the katuday leaves extract does not contain any amounts of flavonoids, saponins, and phenols. While katuday seeds extract contains alkaloids, AA/proteins, carbohydrates, flavonoids, glycosides, steroids, and tannins. Katuday seeds extract does not contain any amounts of saponins and triterpenoids. Discrepancies to the documented constituents may be due to extraction, environment, and age of sample.

This means that katuday leaves contains physiologically active constituents like tannins and triterpenoids which helps in cytotoxic activity. Moreover, katuday seeds contains flavonoids and tannins which also helps in cytotoxic activity. In accordance with this, Deepthi, et. al. (2022) stated that, physiologically active constituents like flavonoids, saponins, tannins, and triterpenoids greatly help in cytotoxic activity. Other bio-active components like alkaloids, AA/proteins, carbohydrates, courmarin, glycosides, phytosterol, phenols, and steroids do not contribute to the cytotoxic capability of the plant.

Table 2. Mean Mitotic Index of Katuday *(Sesbania grandiflora Linnaeus)* using *Allium cepa Assay*

Treatments	Type of Extract	Mitotic Index (%)			Mean
		I	II	III	
T0	Distilled Water	11.88 %	15.46 %	13.86 %	13.73 %
T1	Katuday Leaves	10.38 %	9.82 %	11.57 %	10.59 %
T2	Katuday Seeds	9.51 %	8.48 %	7.14 %	8.38 %

The mean mitotic index of allium cepa assay was shown in table 2. As shown in the table, T0 – distilled water have the highest mean mitotic index with 13.73, second is the T1 – katuday leaves ethanolic extract with a mean mitotic index of 10.59, and T2 - katuday seeds ethanolic extract have the lowest mean mitotic index with 8.38.

According to Ha (2016), high mitotic index might serve as an additional prognostic factor for Hepatocellular carcinoma patients and be a unique predictor of diagnostic decision support.

Moreover, Independent of grade and MIB-1 index, the mitotic index, as determined by pHH3 immunohistochemistry, is a meningioma recurrence predictor (Olar, et. al., 2014).

The increase in a cell's mitotic index indicates that it is rapidly dividing, which is the primary distinction between malignant and normal cells. Genes may be harmed by cancer cells, which also divide rapidly as they spread. *Allium cepa* meristem cells decreased mitotic index can be used as a reliable indicator of a plant's capacity for cytotoxicity. Chromosome structural changes are among the cytotoxic agents.

This implies that T2 which is the katuday seeds is more effective than T1 which is the katuday leaves because it garnered the lowest mean mitotic index.

Table 3. Statistical Analysis on the Mitotic Index of Katuday *(Sesbania grandiflora Linnaeus)* Seeds and Leaves Extract using *Allium cepa Assay*

Indicators	Mean	Alpha Value	P- value	Decision	Interpretation
T1	10.59	0.01	0.06	Accept Ho	No Significance
T2	8.38	0.01	0.06	Accept Ho	No Significance

Statistical Analysis on the mitotic index of *Allium cepa* assay was shown in table 3. Using T-test for Independent Samples, it was found out that the calculated P – value for T1 and T2 which is 0.06 is greater than the alpha value of 0.01, therefore, the null hypothesis is accepted implying that there is no significant difference among the treatments.

The formulated treatments, T1 - katuday leaves ethanolic extract and T2 – katuday seeds ethanolic extract are proven to exhibit cytotoxic capabilities as shown in the phytochemical analysis. Hence any part of the plant may be effective provided and followed by a thorough validation of being a cytotoxic agent.

In connection to this, a study conducted by Patil (2022), stated that the entire katuday ***(****Sesbania grandiflora Linnaeus****)*** plant, as well as the leaves, bark, roots, seeds, flowers, and fruits, include medicinal properties. The significance of katuday as a nutraceutical and the presence of certain phytochemicals including alkaloids, anthraquinone, flavonoids, steroids, phytosterol, terpenoids, tannins, catechin, etc. in it are both supported by a large body of study.

Moreover, Patil (2022) also stated that, katuday ***(****Sesbania grandiflora Linnaeus****)*** is said to have essential therapeutic qualities as well. It is well recognized for its rich source of minerals, vitamin A, calcium, carbs, and phosphorus. It also has many helpful effects against bacterial infection, inflammation, tumor, etc. In lymphoma and colon cancer cells, katuday **(***Sesbania grandiflora Linnaeus***)** flower extract slows cell proliferation, induces apoptosis, and reduces tumor volume. Acrylonitrile is one of the isolated compounds from katuday **(***Sesbania grandiflora Linnaeus***)** leaves which helps in anti-proliferative and antitumor activity.

All in all, katuday seeds ethanolic extract yieled the lowest result in terms of mitotic index which means it has the highest cytotoxic capability. Although, any part of the katuday plant can be used as a cytotoxic agent following the results of this study and considering the study of Patil (2022).

CHAPTER IV

CONCLUSION AND RECOMMENDATIONS

Following the results and findings, the following conclusions were derived. The katuday ***(****Sesbania grandiflora Linnaeus****)*** leaves and seeds have physiologically active constituents like flavonoids, saponins, tannins, and triterpenoids which helps in cytotoxic activity as shown on table one (1). Moreover, T2 which is the katuday seeds extract yielded the lowest result in terms of the mean of the mitotic index which implies that this treatment has the highest cytotoxic capability. Although T1 - katuday leaves extract and T2 - katuday seeds extract has no significant difference. Therefore, any part of the plant can be used as a cytotoxic agent.

Further improvement may be done to this study. A follow-up study can be conducted using other parts of the plant, another type of plant, and an increase of trials. Moreover, additional study shall be conducted regarding other uses of the plant. Other parts of the plant shall also be tested as to effectiveness to combat other related diseases. Future researchers may also develop a product which contains katuday seeds and leaves extract which can be used as a cytotoxic drug.

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APPENDICES

Appendix Table 1. Phytochemical Analysis of Katuday *(Sesbania grandiflora L.)*

Seeds and Leaves

Sr. No.	Phytochemical constituents	Leaves	Seeds
1.	Alkaloids	+	+
2.	AA / Proteins	-	+
3.	Carbohydrates	+	+
4.	Coumarin	-	-
5.	Flavonoids	-	+
6.	Glycosides	-	+
7.	Phytosterol	-	-
8.	Phenols	-	-
9.	Saponin	-	+
10.	Steroids	+	+
11.	Tannins	+	+
12.	Triterpenoids	+	-

*Legend: (-) negative; (+) positive.

Appendix Table 2. Table 2. Mean Mitotic Index of Katuday *(Sesbania grandiflora L.)* using *Allium cepa Assay*

Treatments	Type of Extract	Mitotic Index (%)			Mean
		I	II	III	
T0	Distilled Water	11.88 %	15.46 %	13.86 %	13.73 %
T1	Katuday Leaves	10.38 %	9.82 %	11.57 %	10.59 %
T2	Katuday Seeds	9.51 %	8.48 %	7.14 %	8.38 %

Appendix Table 3. Statistical Analysis on the Mitotic Index of Katuday *(Sesbania grandiflora L.)* Seeds and Leaves Extract using *Allium cepa Assay*

Indicators	Mean	Alpha Value	P- value	Decision	Interpretation
T1	10.59	0.01	0.06	Accept Ho	No Significance
T2	8.38	0.01	0.06	Accept Ho	No Significance

T-test for Independent Samples

	T1	T2
Mean	10.59	8.37666666666667
Variance	0.7987	1.41223333333333
Observation	3	3
Hypothesized Mean Difference	0	
df	4	
T-stat	2.57821795349109	
P-value	0.0614429271084669	
Critical Value	4.60409487134999	

Appendix Table 4. Dividing Cell, Total Cell, and Mitotic Index of Each Treatments

Treatments	Dividing Cell	Total Cell	Mitotic Index	Mean Mitotic Index
T0R1	117	985	11.88%	13.73%
T0R2	149	964	15.46%	
T0R3	125	902	13.86%	
T1R1	93	389	23.91%	25.77%
T1R2	79	267	29.59%	
T1R3	86	361	23.82%	
T2R1	64	673	9.51%	8.38%
T2R2	52	613	8.48%	
T2R3	47	658	7.14%	

Division of La union
REGIONAL SCIENCE HIGH SCHOOL for REGION I
Maria Cristina East, Bangar, La Union

RECORDS OFFICE
DMMMSU - NLUC
RECEIVED
DATE 04/13/2023

April 18, 2023

Dr. Junifer Rey E. Tabafunda
The Chancellor
Don Mariano Marcos Memorial State
University–North La Union Campus

Sir:

Greetings in the name of the Lord!!!

We are students from the **Regional Science High School for Region I**, and we are pleased to inform you that we are currently conducting our experimentation for our research study in partial fulfillment of the requirements in **Science Investigatory Project/Research Project**. The researchers are currently studying the ***Cytotoxic Capabilities of Katuday (Sesbania grandiflora) Flowers, Seeds, and Leaves Extract using Allium cepa Assay***. In connection with this, the researchers would like to conduct experimentation in your school.

In this regard, we humbly ask for your assistance and approval knowing fully that your institution is accredited in this endeavor. Rest assured that the data to be gathered will be utilized according to their educational purposes only.

We are looking forward to a positive response regarding this humble request in the name of noble research. Thank you.

Respectfully yours,

Cantos, Allyssa Mae C.

Dato Kim Jeyeon A.

Valdez, Daphne Kate V.
The researchers

DR. RAUL SAMPLEZ
PLEASE ACCOMMODATE THIS REQUEST. LET US KNOW OUR ASSISTANCE TO THE RESEARCHERS.

Rowel P. Lucina,MAN,RN,LPT *09459623148/rowel.lucina@deped.gov.ph*
Research Adviser

PLATES

Plate 1. Katuday (*Sesbania grandiflora L.)* Leaves

Plate 2. Katuday *(Sesbania grandiflora L.)* Seeds

Plate 3. Katuday *(Sesbania grandiflora L.)* Leaves Extract

Plate 4. Katuday (Sesbania grandiflora L.) Seeds Extract

Plate 5. Microscope

Plate 6. Materials and Equipment

Plate 7. ***Allium cepa*** **Assay**

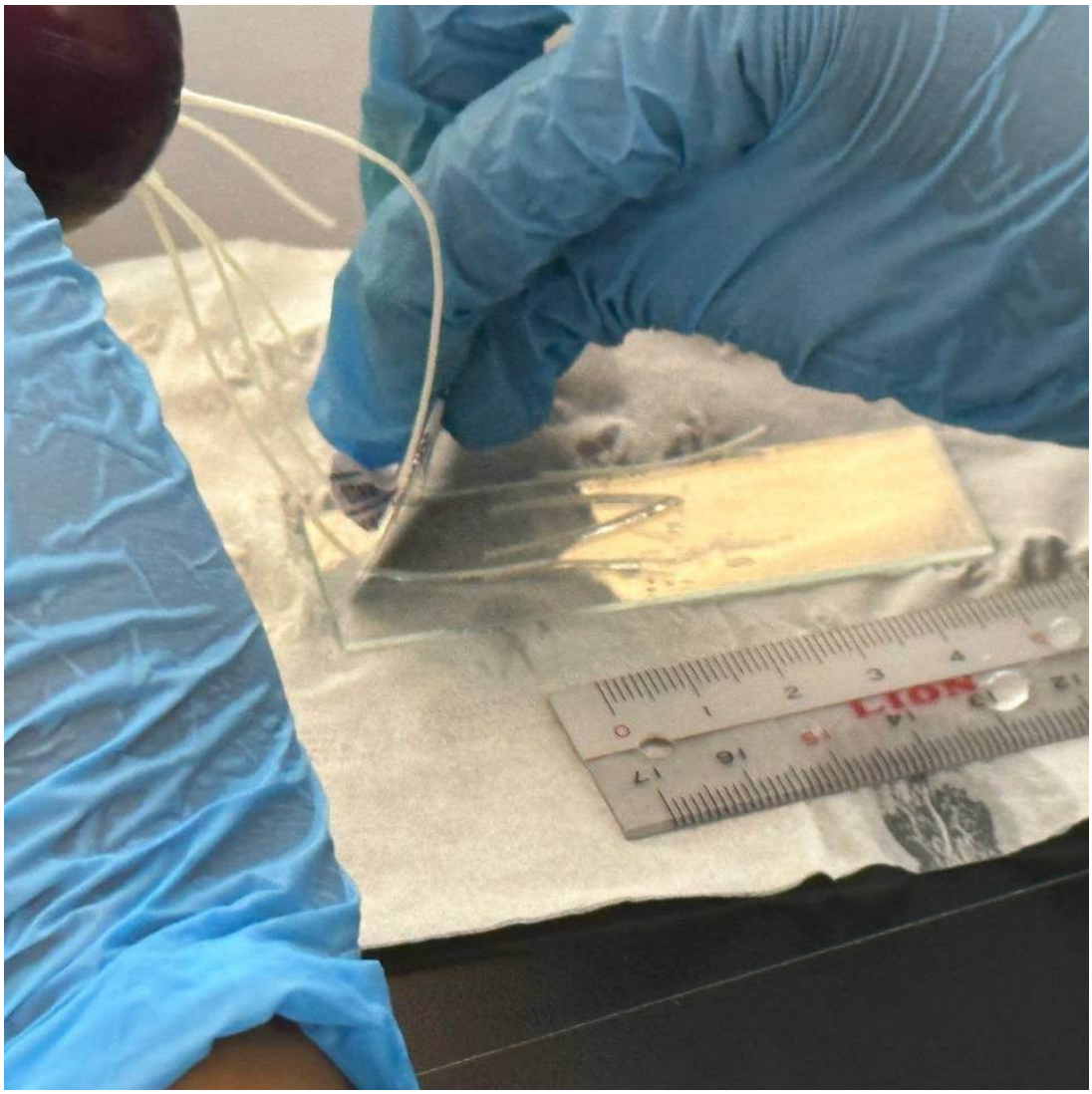


Plate 8. Cutting of two (2) cm onion root tip

Plant 9. Submerging two (2) cm to the extract

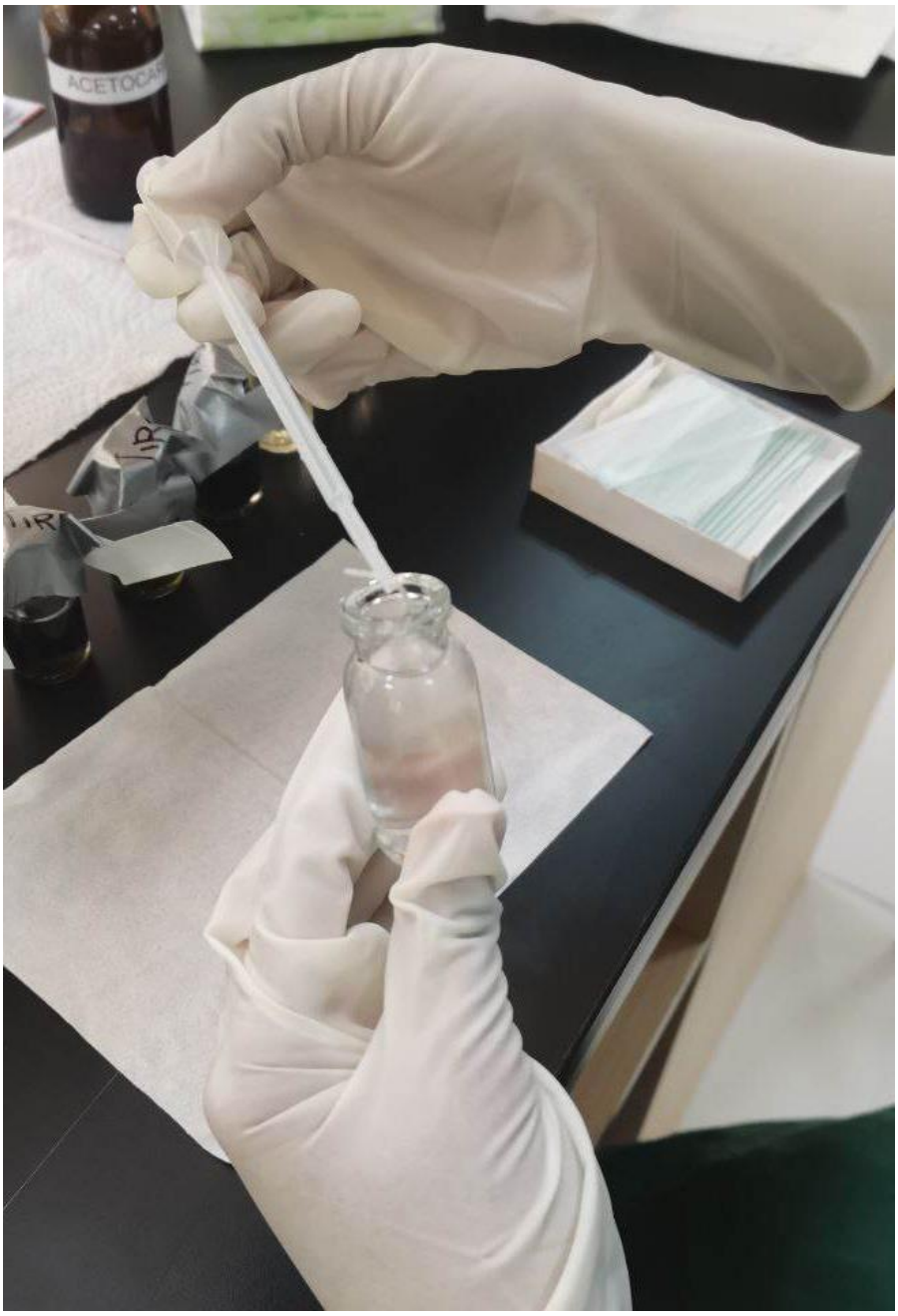


Plate 10. Removing the root tips on the container with extract

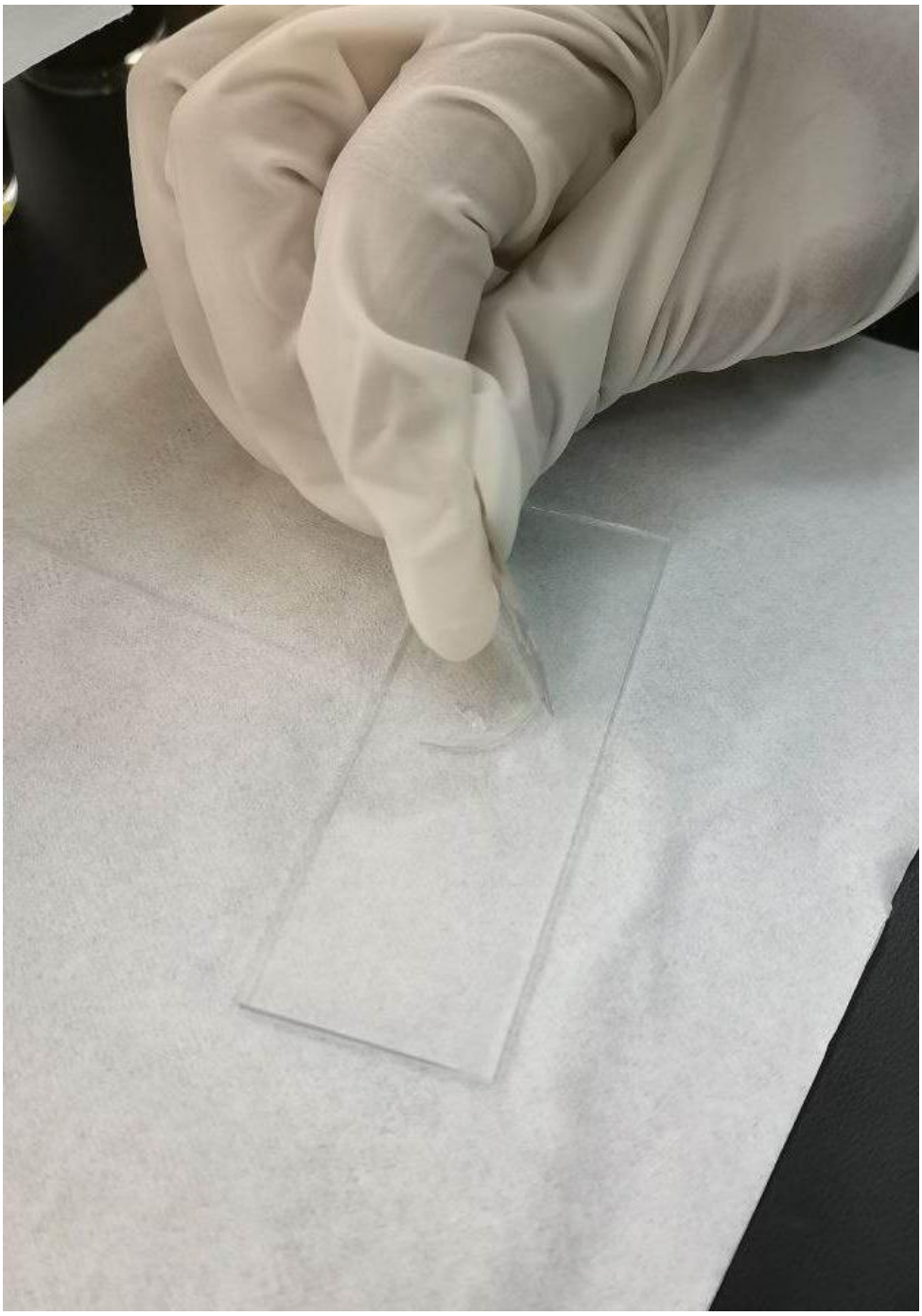


Plate 11. Cutting the very tip of the root

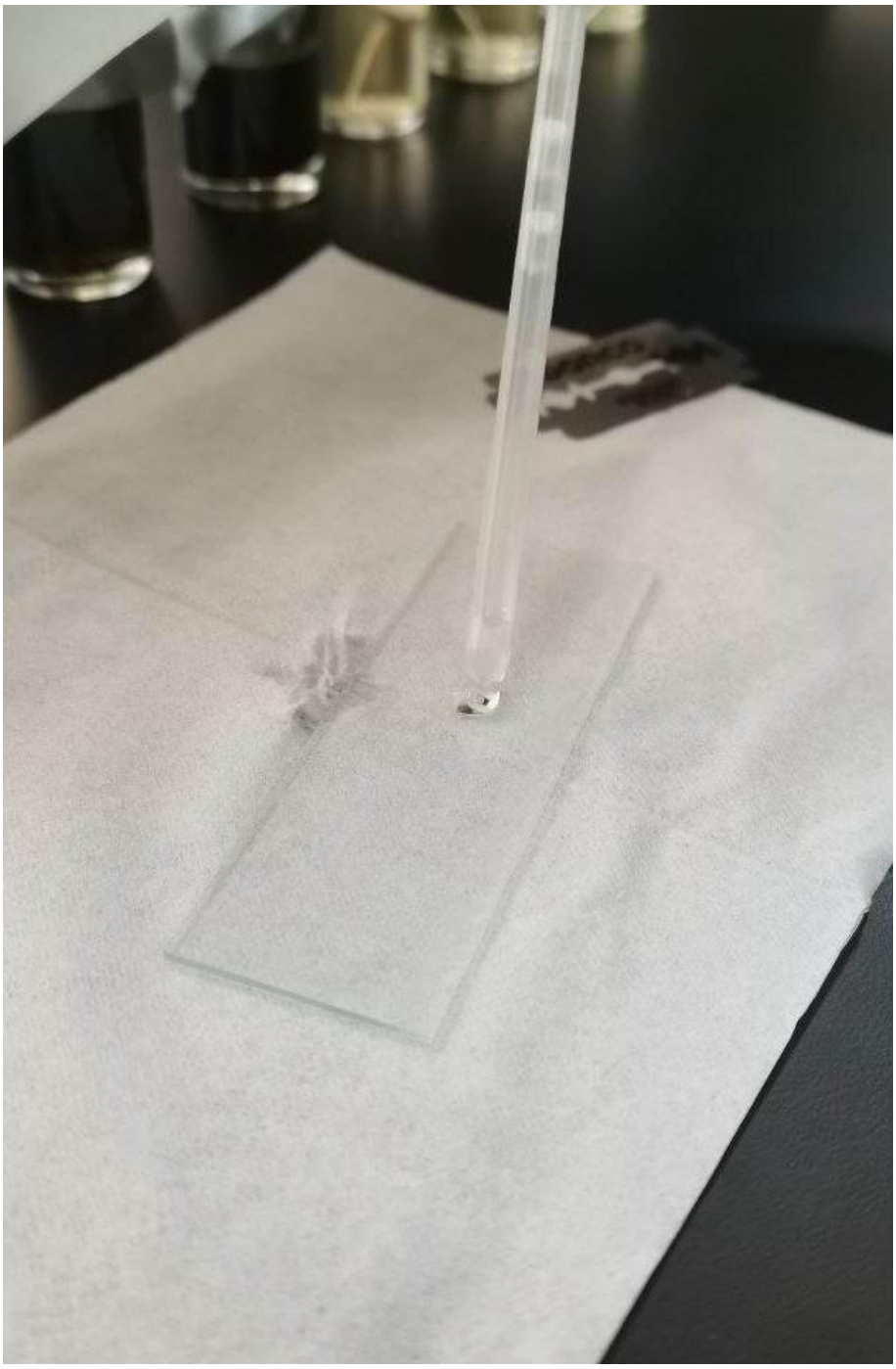


Plate 12. Dropping of 1M HCl

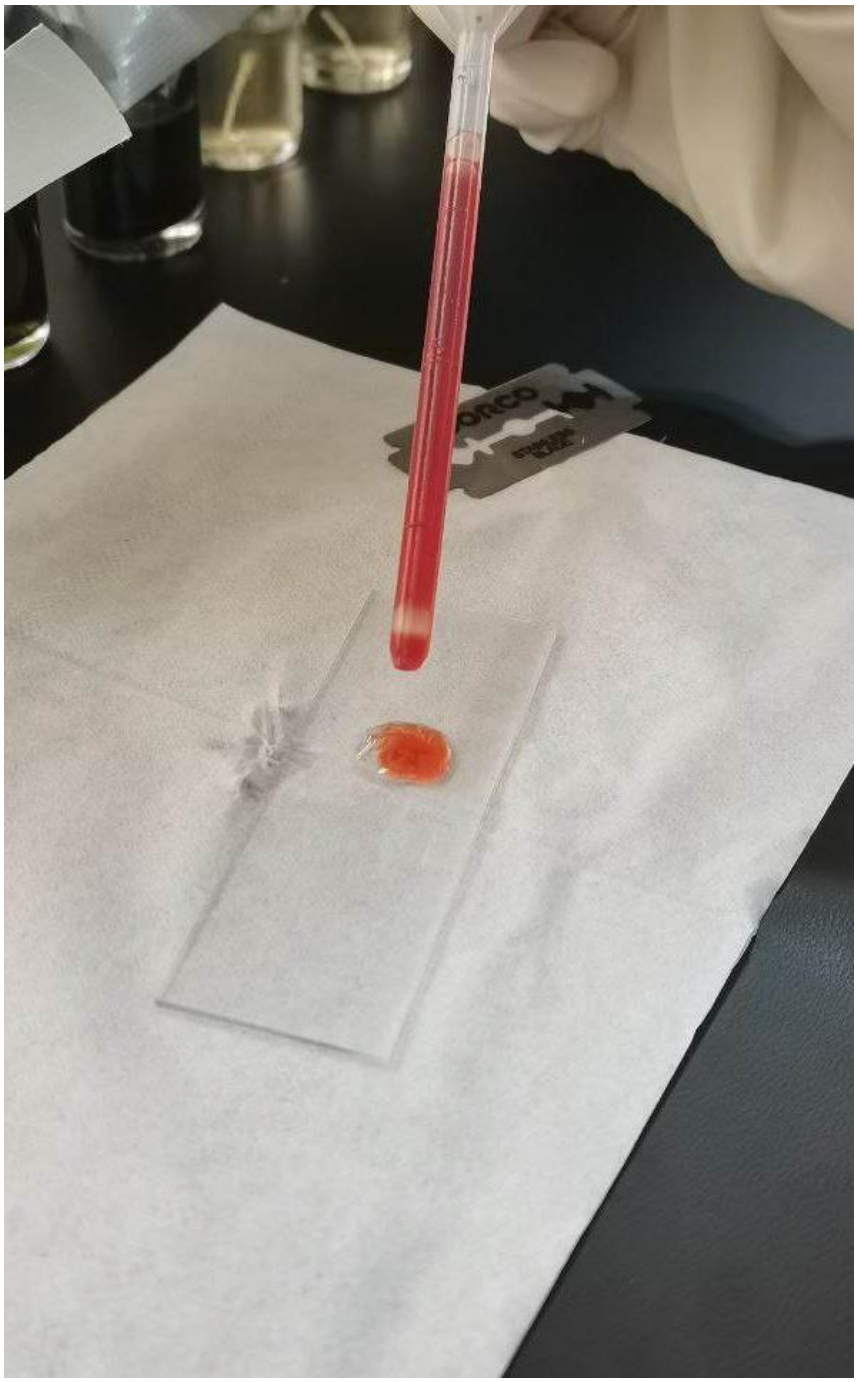


Plate 13. Dropping of acetocarmine

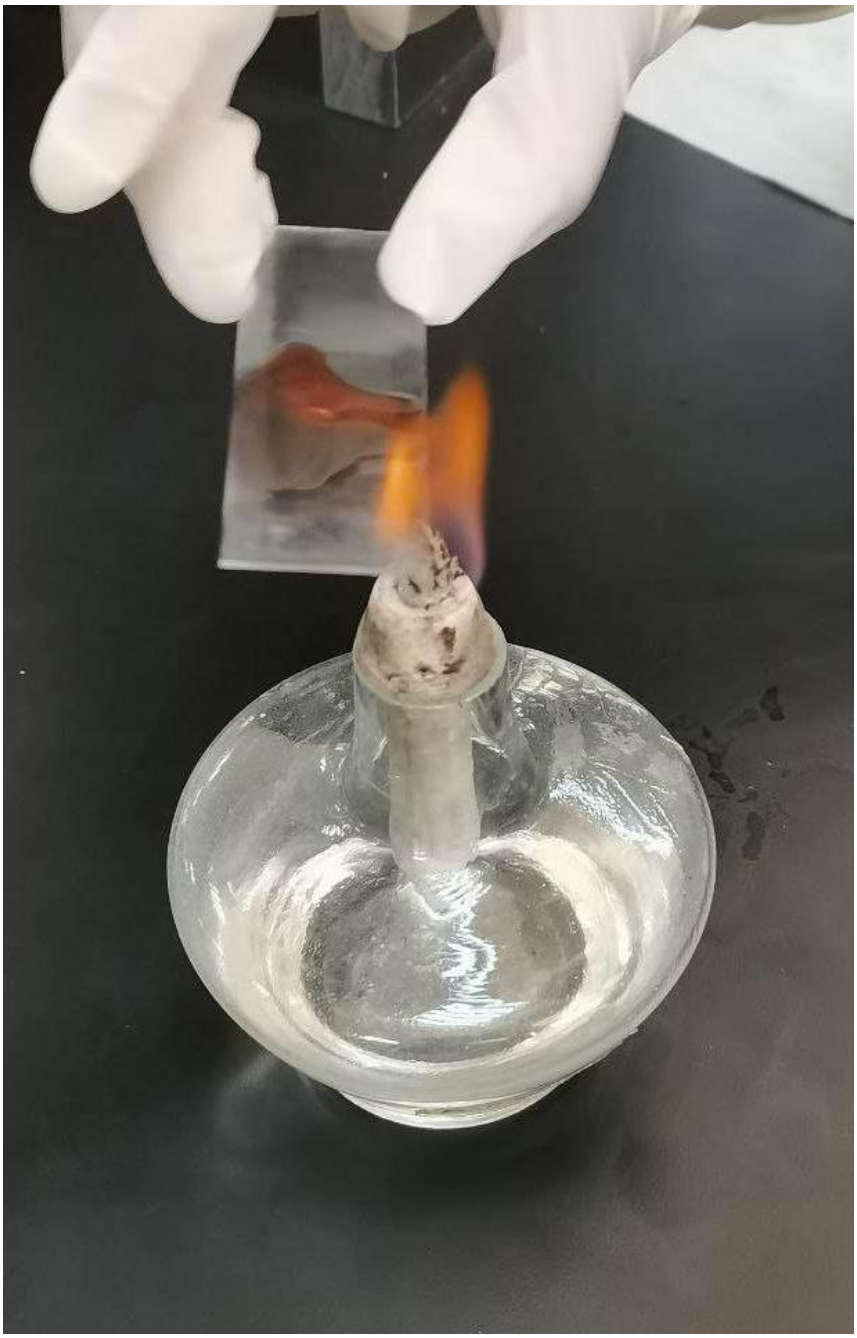


Plate 14. Heating the slide

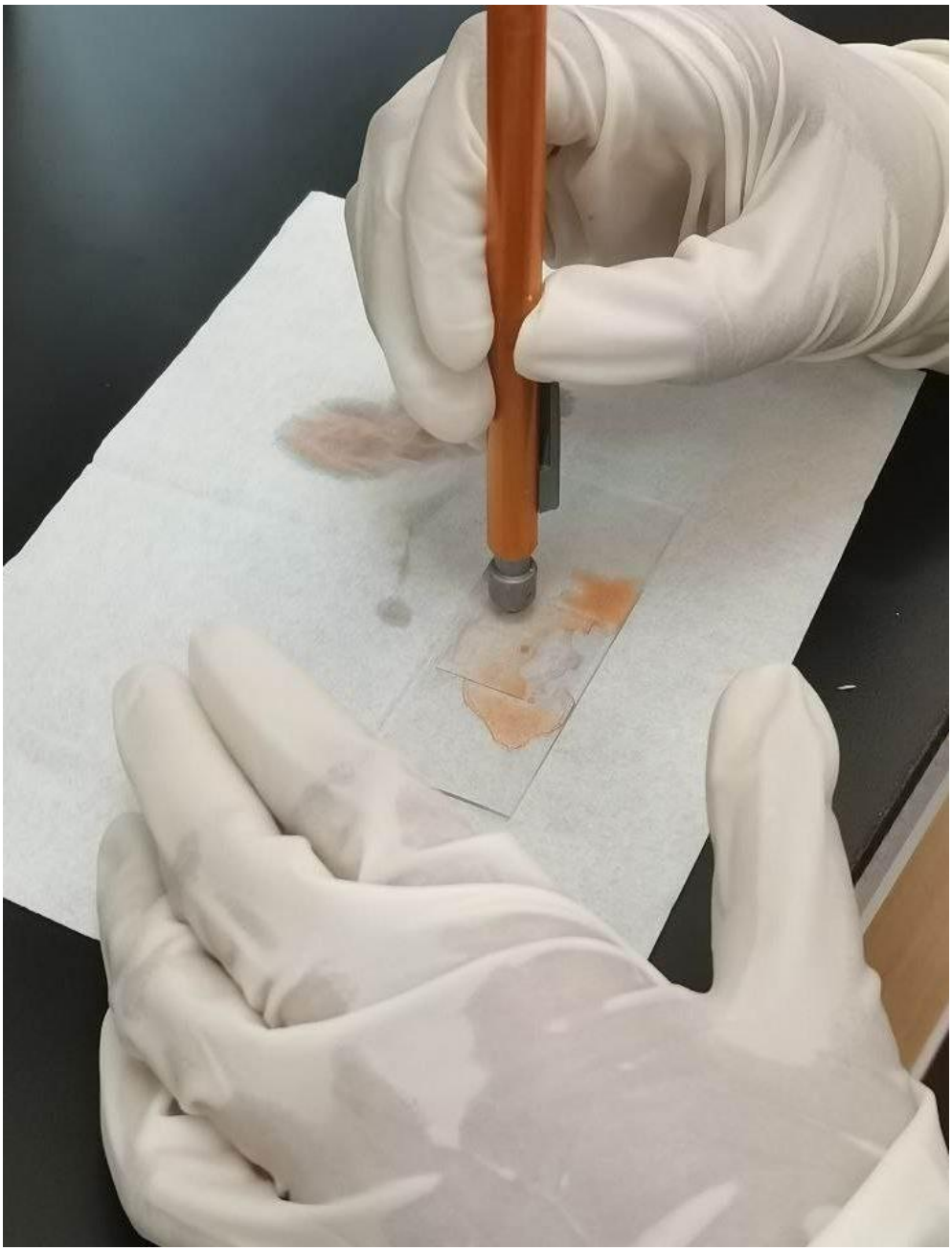


Plate 15. Blotting the excess stain and pressing the root tip

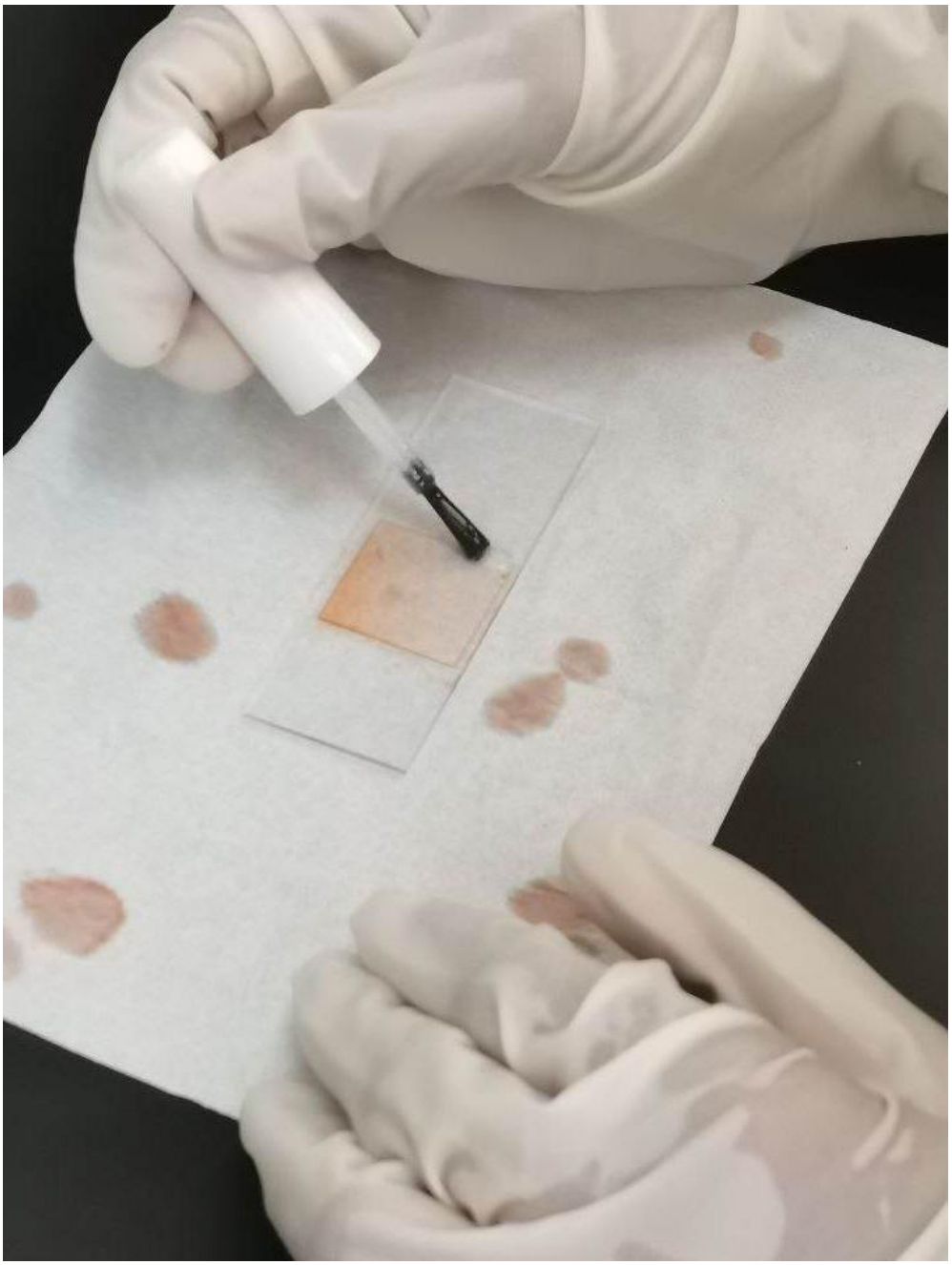


Plate 16. Fixing the slide using nail polish

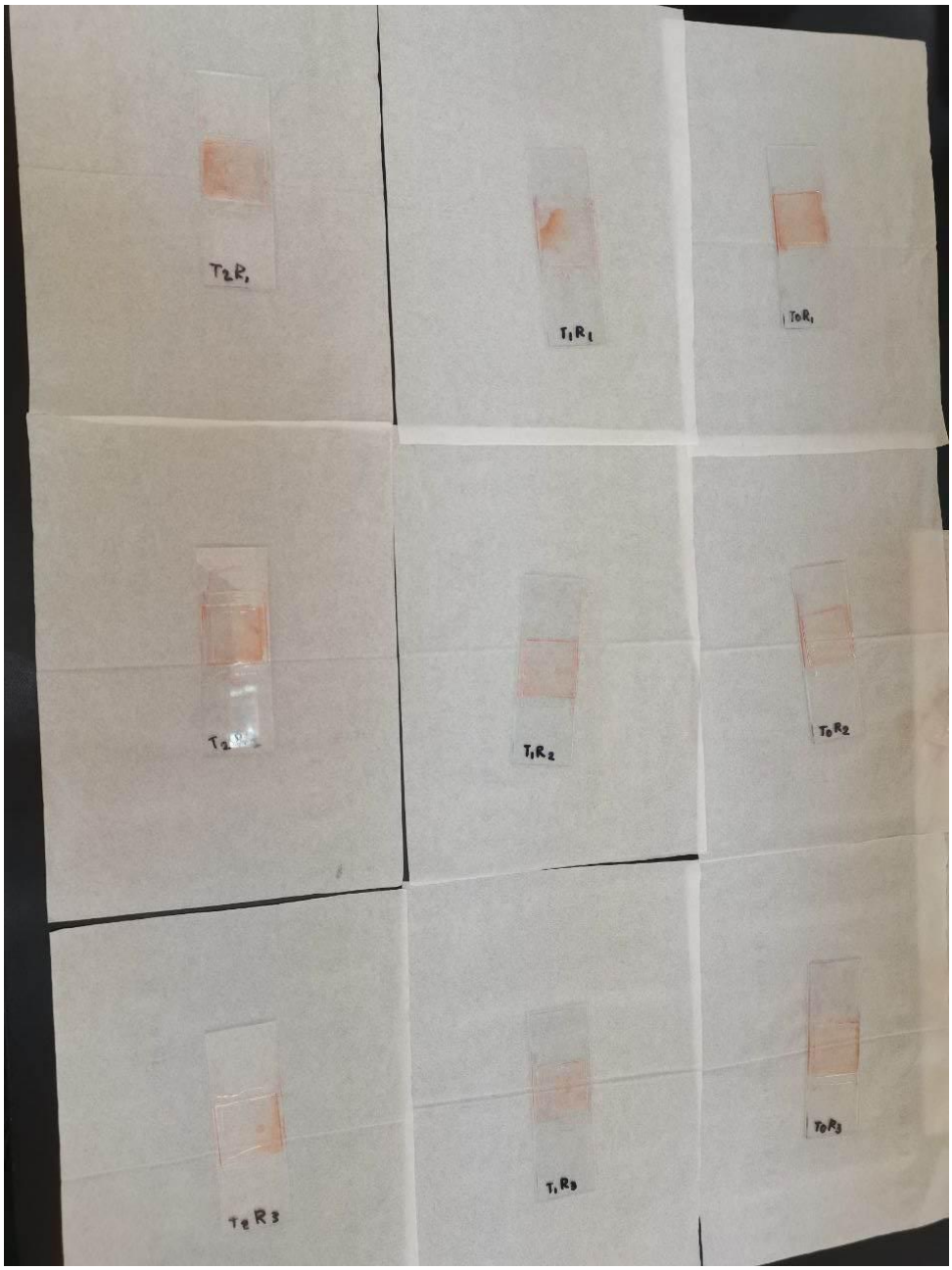


Plate 17. The treatments

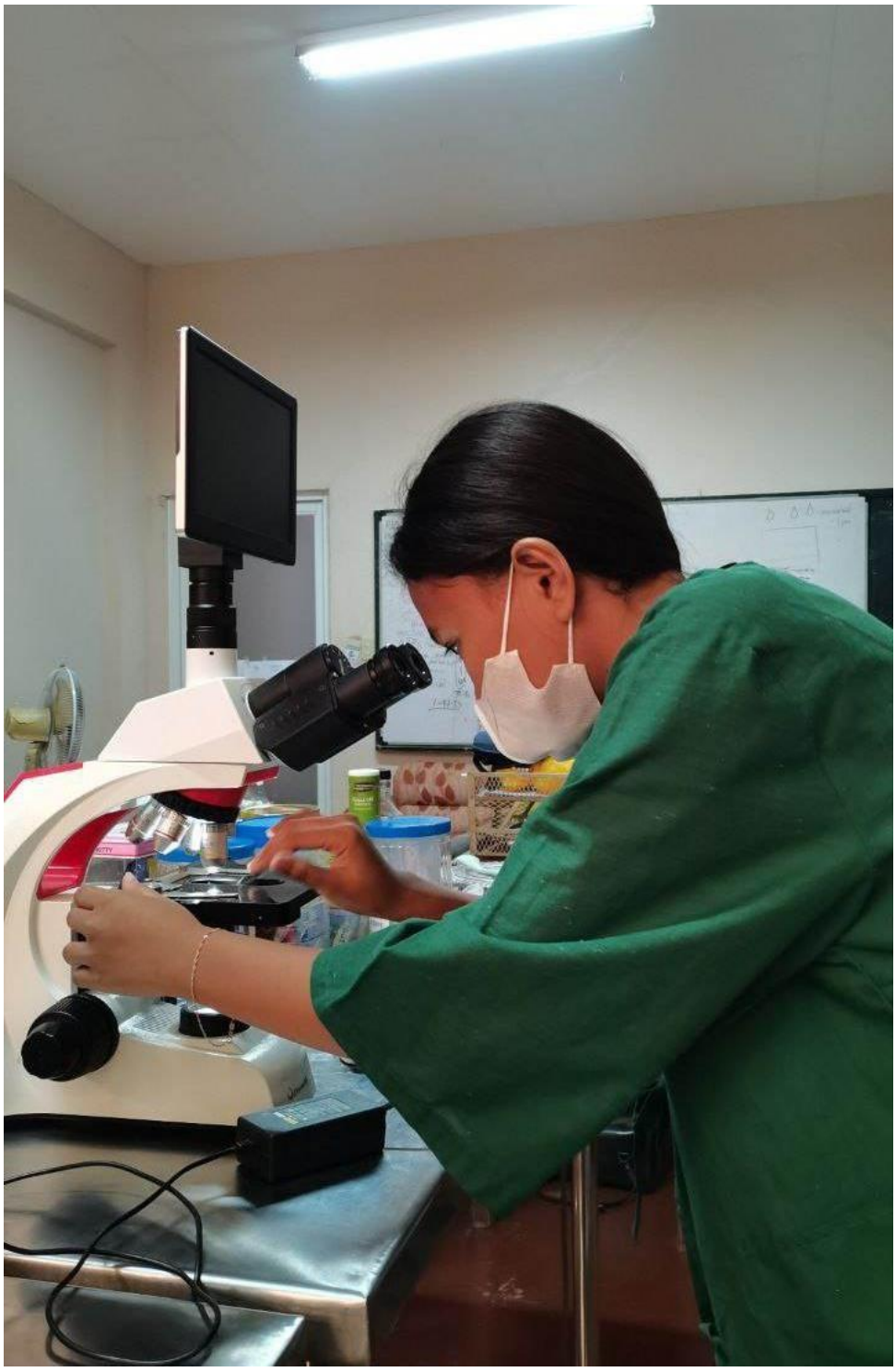


Plate 18. Observing the sample in the microscope

Plate 19. Replicate one (1) T0 – 100% distilled water

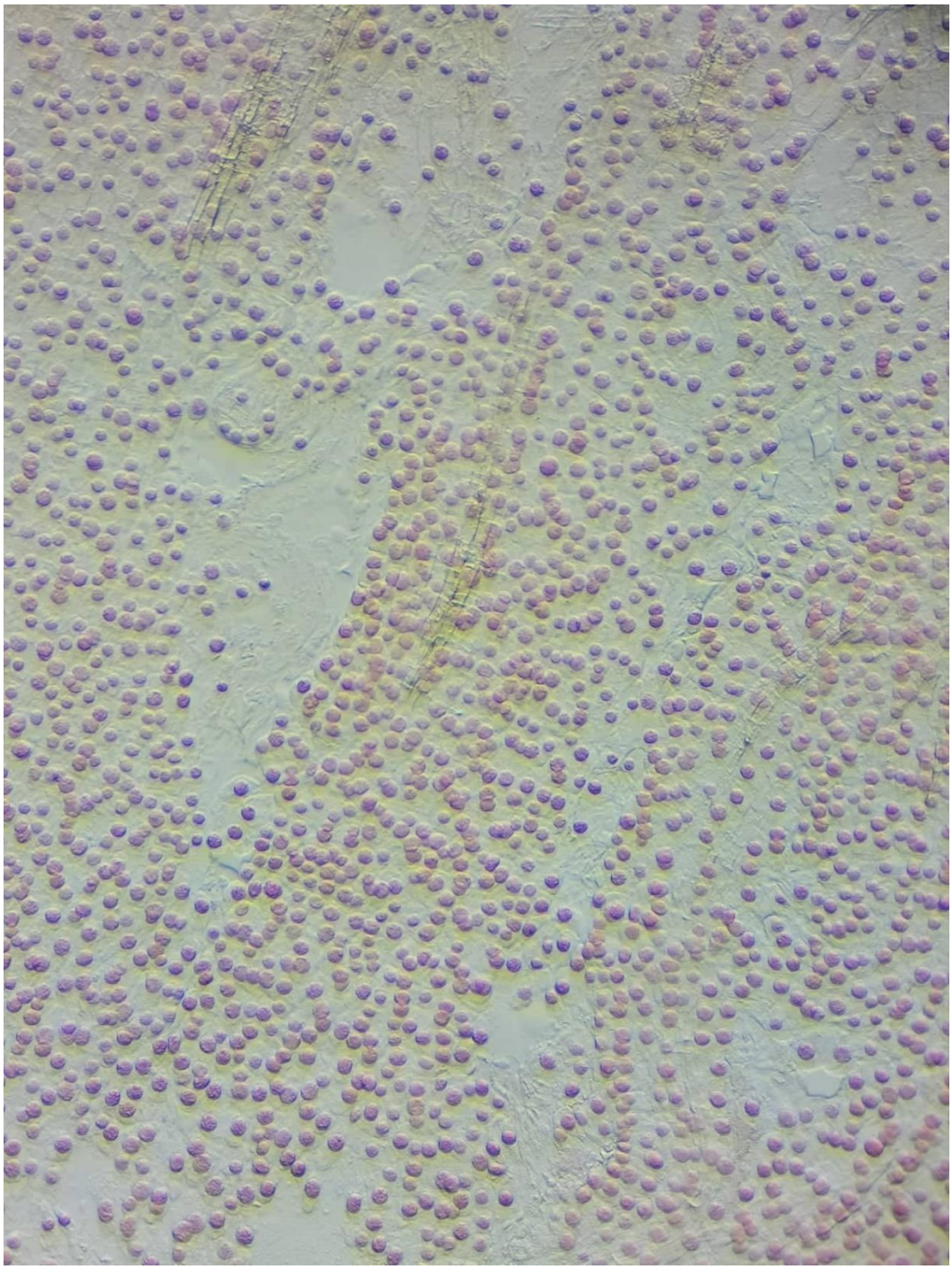


Plate 20. Replicate two (2) T0 – 100% distilled water

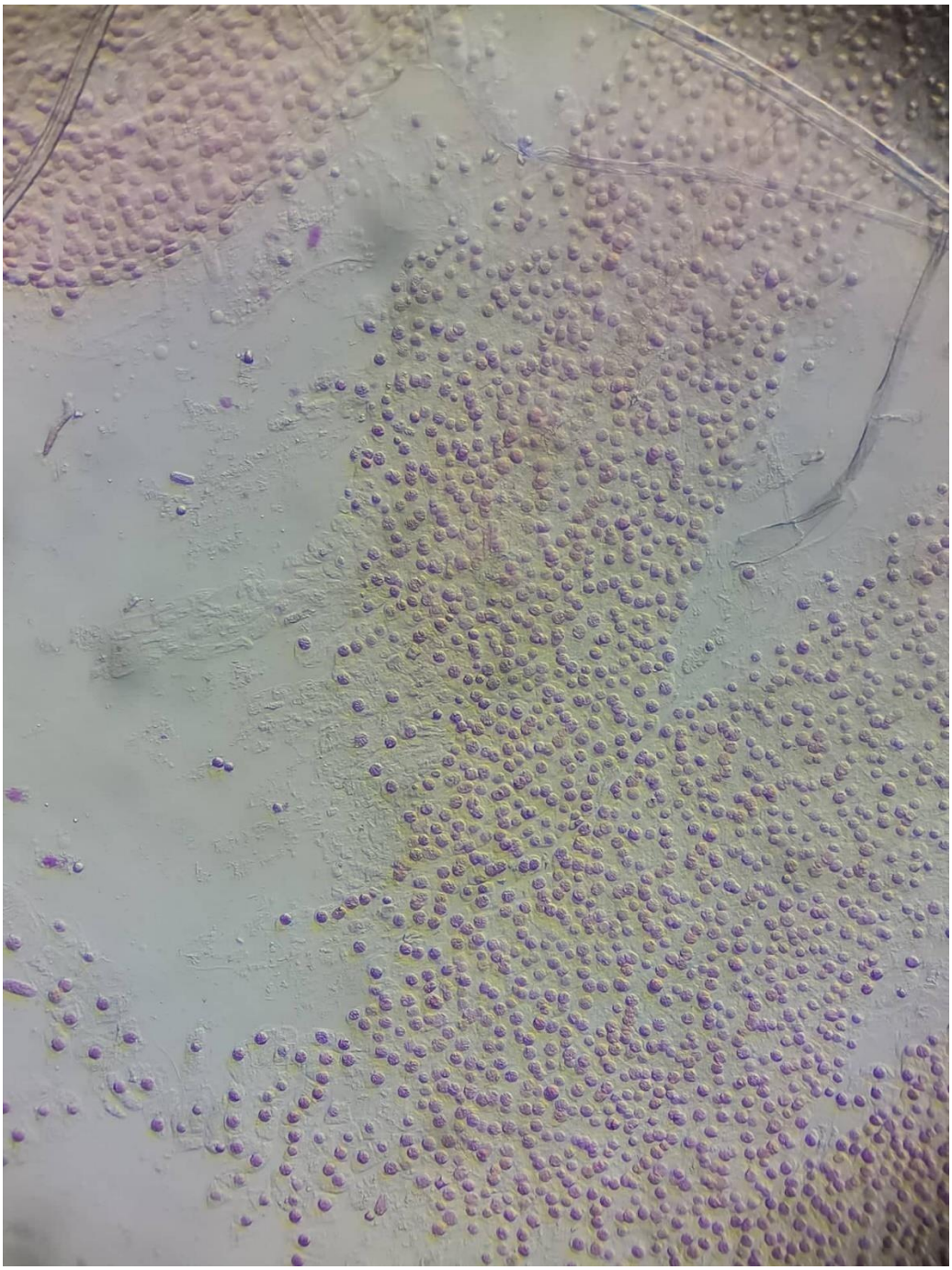


Plate 21. Replicate three (3) T0 – 100% distilled water

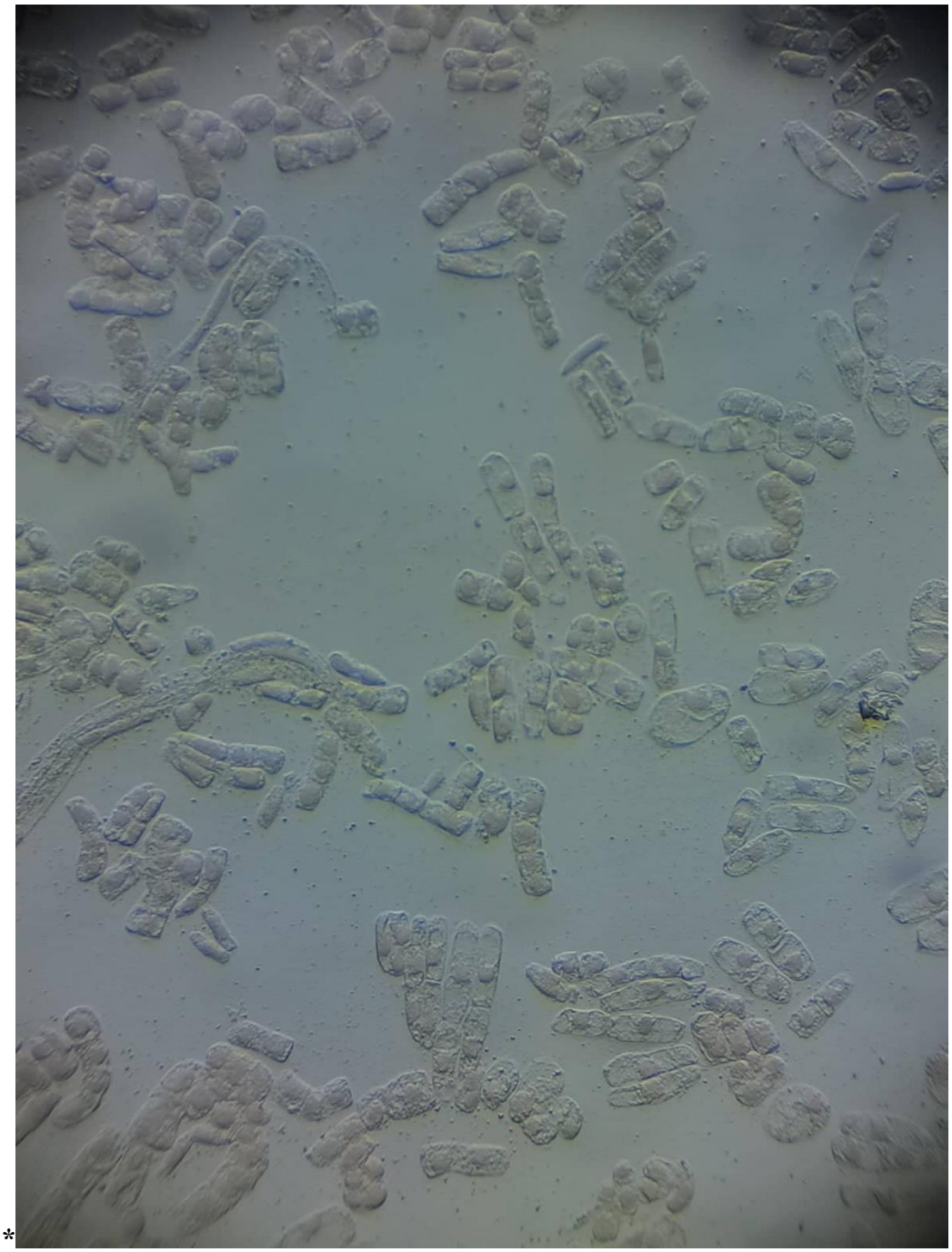


Plate 22. Replicate one (1) T1 - 100% katuday leaves extract

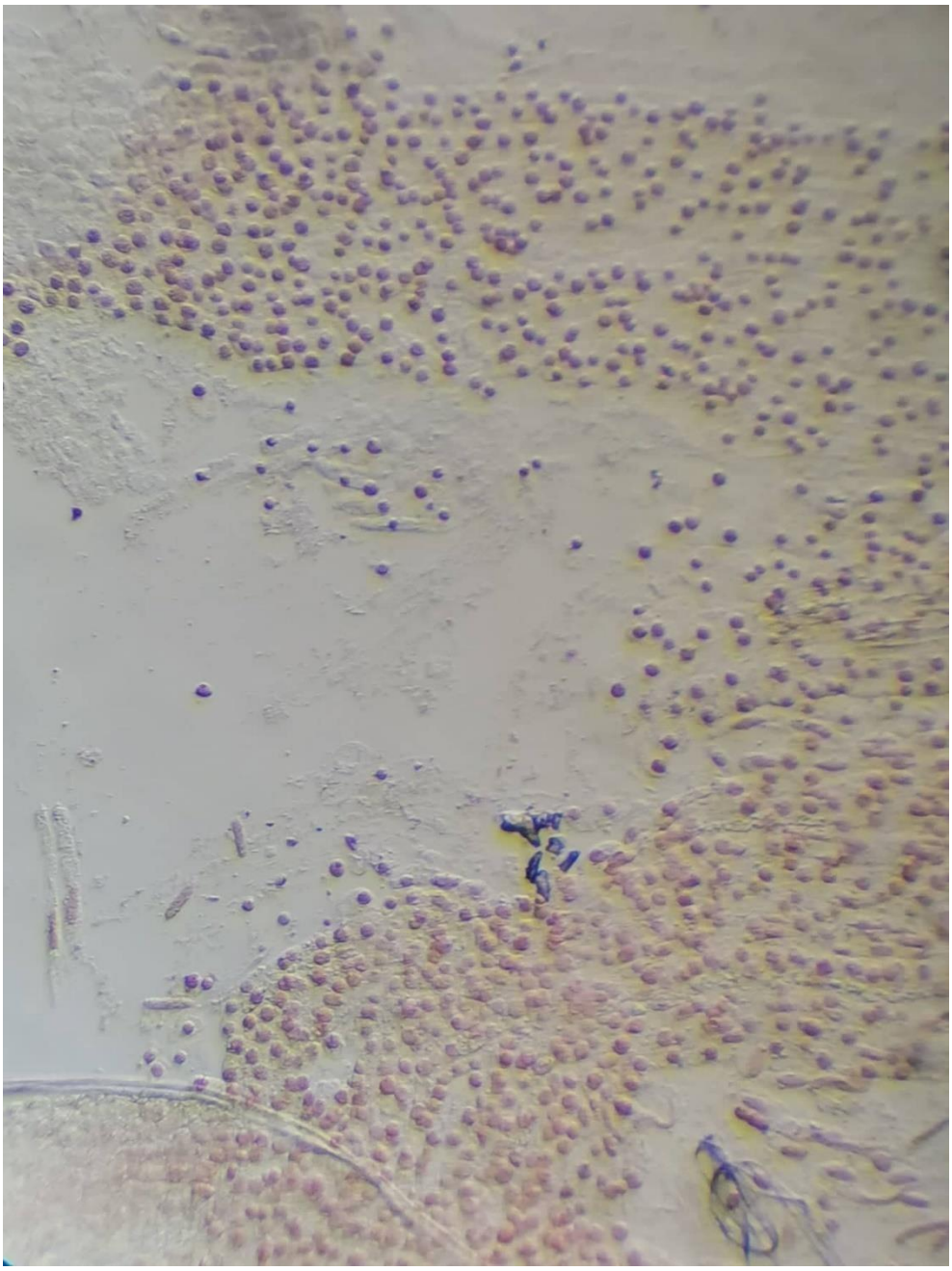


Plate 23. Replicate two (2) T1 - 100% katuday leaves extract

Plate 24. Replicate three (3) T1 - 100% katuday leaves extract

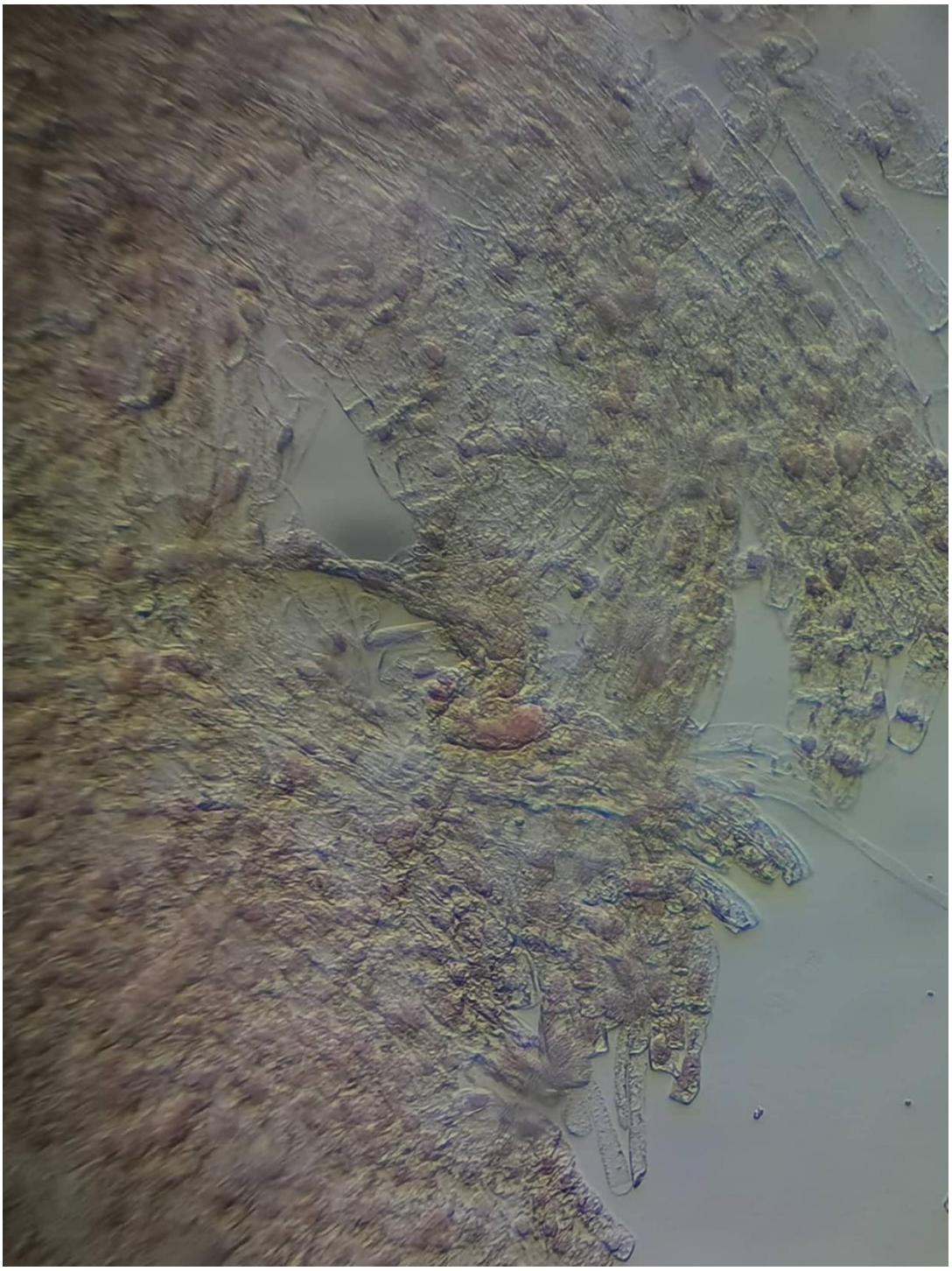


Plate 25. Replicate one (1) T2 – 100% katuday seeds extract

Plate 26. Replicate two (2) T2 – 100% katuday seeds extract

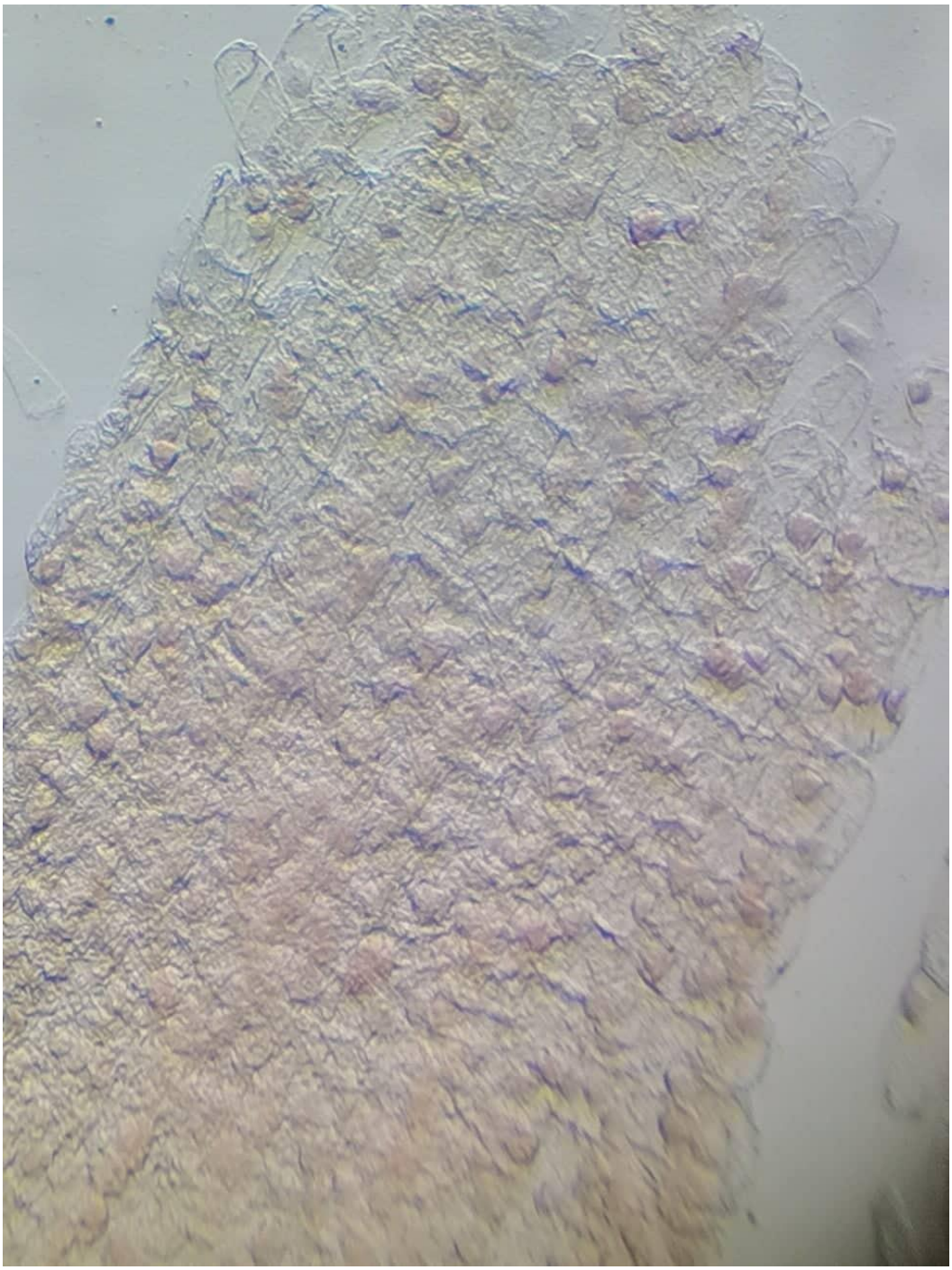


Plate 27. Replicate three (3) T2 – 100% katuday seeds extract

DATA BOOK

Activities	Date started	Date accomplished	Description
Choosing a topic and identifying the problem.	September 14, 2022	September 16, 2022	The researchers started to think of existing topics and problems to venture on.
Title proposal	September 13,2022	September 13, 2022	The researchers proposed their studies that was acquired through defending the title of the chosen topic.
Making the write up of Chapter I	September 24, 2022	September 26, 2022	The researchers searched for related literatures to come up of a SOP and make the chapter I.
Improving Chapter I	September 26, 2022	September 26, 2022	The researchers improved the content of their Chapter I.
Checking of SOP	October 09, 2022	October 09, 2022	The research adviser checked for the SOP of the study.
Checking of Chapter I	October 12, 2022	October 12, 2022	The research adviser suggested some suggestions refarding the Chapter I.
Making the write yo of Chapter II	November 26, 2022	November 30, 2022	The researchers made the Chapter II to be reflected on the experiment. They also searched for protocols and RRLs.

Gathering of materials	February 1, 2023	February 1, 2023	The researcher gathered plant sample from their backyard for the plant extraction.
Plant extraction	February 07, 2023	February 07, 2023	Plant sample were submitted at Lorma
			Colleges for the ethanolic extraction.
Experimentation	March 15, 2023	March 15, 2023	The experimentation were executed in RSHS for R1 laboratory.
Repeat the experimentation	April 17, 2023	April 18,2023	The researchers prepared to do the experimentation again.
Experimentation	April 28, 2023	May 04, 2023	The researchers conducted the experiment at DMMMSU NLUC and gathered data.
Making the write up of Chapter III and IV	May 18, 2023	May 19, 2023	The researchers made the write uo of results and discussion from the gathered data.
Making of display board	May 19, 2023	May 19, 2023	The researchers created the display board for the oral defense.
Proofreading Chapter I-IV	May 20, 2023	May 20, 2023	The researchers proofread and edited their manuscript.

DATE 09/13/2022

Proposal (Title)

- Acaricidal Activity of Annato (Bixa orellana) seeds extract against sarcoptic Mange
- cytotoxic capabilities of Katuday (sesbania grandiflora) flowers extracts using Allium cepa Assay.

→ Approved

Final Title:
cytotoxic capabilities of Katuday (sesbania grandiflora L.) leaves and seeds extract using Allium cepa Assay

09/24/2022

Chapter 1:
20 RRL (10 yrs)
definition of terms
referencs
plagiarism checker

09/26/2022

Enhancing chapter 1

DATE 10/09/2022

checking of SOP:

This study aims to determine the cytotoxic capabilities of katuday (Sesbania grandiflora) seeds and leaves using allium cepa assay. specifically it seeks to answer the following sub-problems:

1) What bio-active components of katuday seeds and leaves extract can help in reducing cancer cells?
2) which among the treatments yields the best result?
   a) T0 = 100% distilled water
   b) T1 = 100% katuday leaves extract
   c) T2 = 100% katuday seeds extract
3) Is there a significant difference among the treatments in terms of mitotic index?

10/12/2022

checking of chapter 1

↳ ppt & chap 1 (print)

DATE 11/26/2022

Chapter 2 : Methodology

Ethical considerations

Throughout the study, the researchers will exemplify honesty fairness and intergrity. The findings of this exprimint will be reported accurately and without bias. Proper protocols will be followed in conducting the experimentation. All the processes that will be conducted will be based on past studies connected to the topic.

11/30/2022

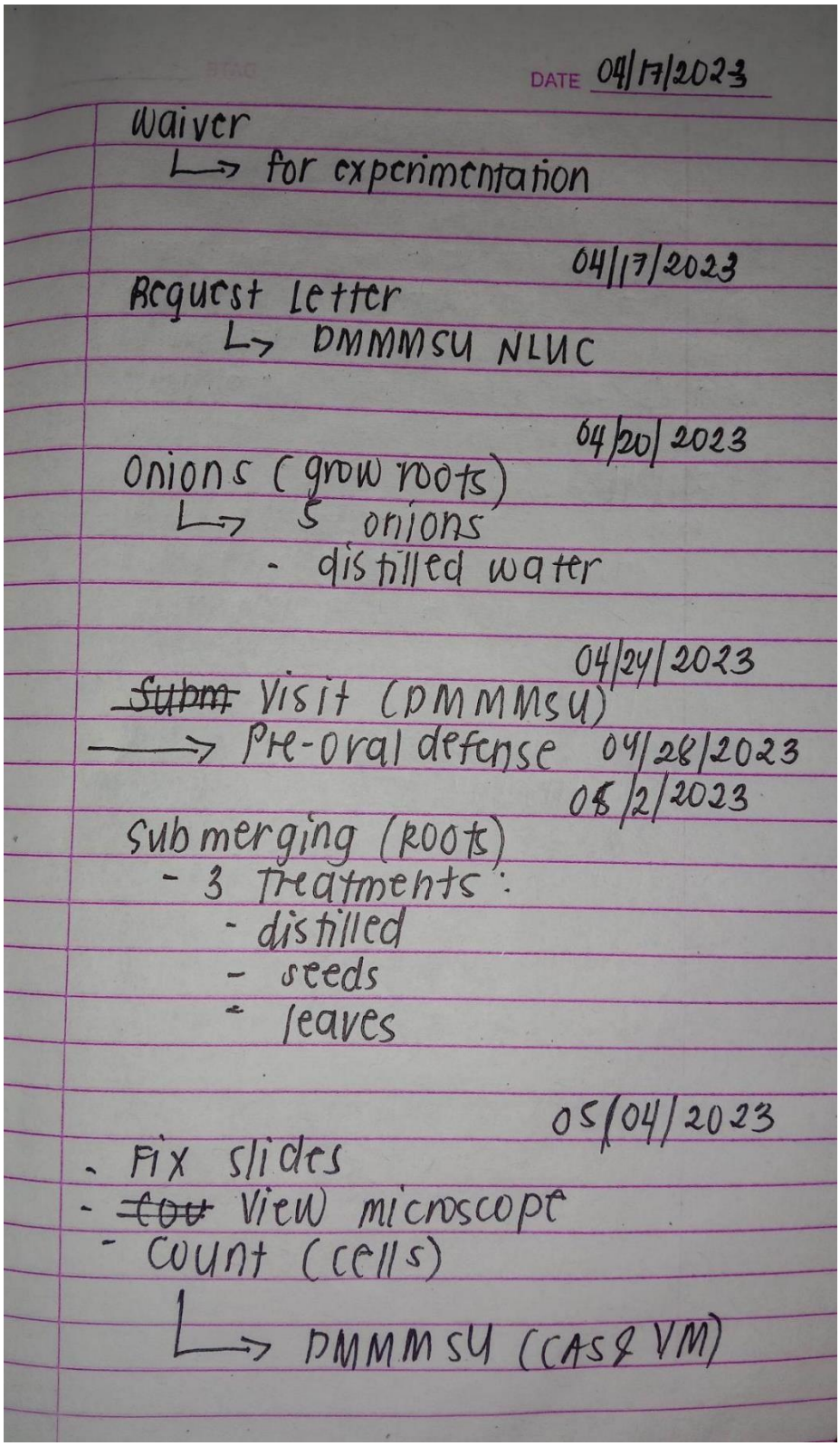
Chap 2 → improvement

02/07/2023

~~Phytochem~~

Extraction

→ Lorma Colleges
- Ethanolic
- 2 samples
Php 2,000



DATE 04/17/2023

Waiver
↳ for experimentation

04/17/2023

Request letter
↳ DMMMSU NLUC

04/20/2023

Onions (grow roots)
↳ 5 onions
- distilled water

04/24/2023

~~Subm~~ Visit (DMMMSU)
→ Pre-oral defense 04/28/2023

05/2/2023

Submerging (roots)
- 3 Treatments:
  - distilled
  - seeds
  - leaves

05/04/2023

- Fix slides
- ~~cou~~ View microscope
- count (cells)
  ↳ DMMMSU (CAS & VM)

DATE

05/06/2023

Count (cells)

05/16/2023

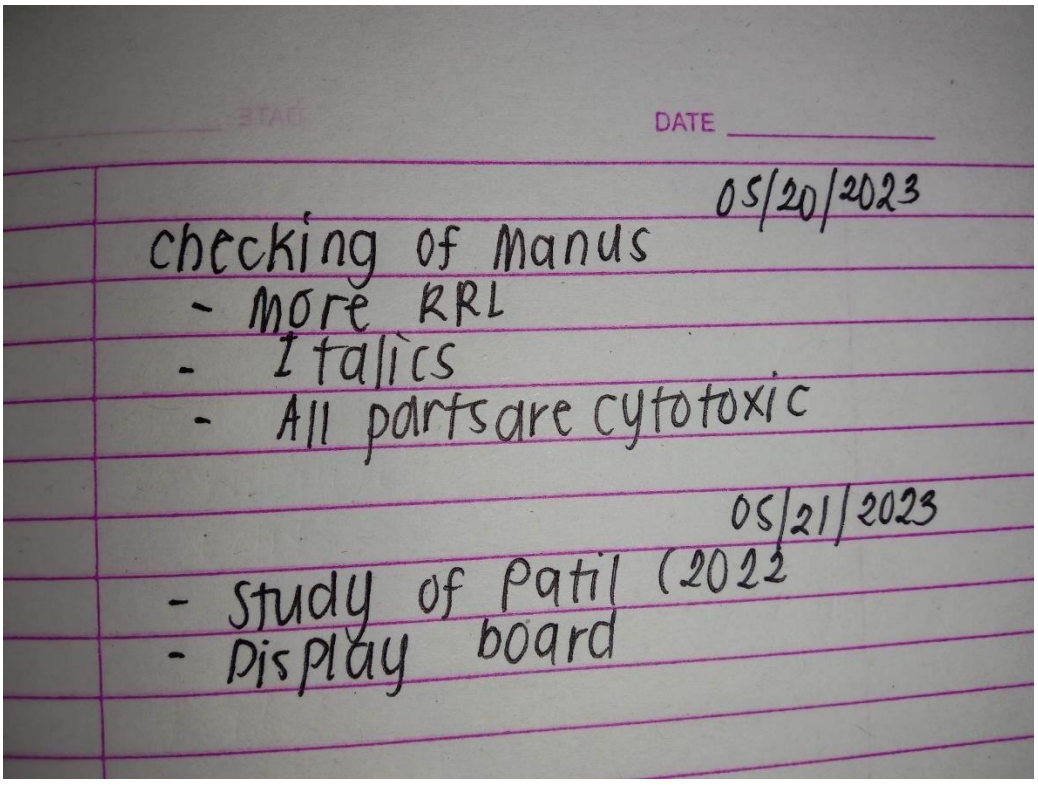
- Display board
- Finalize
- Edit

05/19/2023

- Edit (Manus)
- More RRL
- Tables
- Statistics

T-test for Independent samples
p-value = 0.060088
Critical value = 6.96455
Alpha = 0.01
Mean : (MI)
T1 : 10.59 %
T2 : 8.38 %

NO SIGNIFANT DIFFERENCE
ACCEPT Ho


DATE
05/20/2023
checking of manus
- more RRL
- Italics
- All partsare cytotoxic
05/21/2023
- study of Patil (2022
- Display board

CURRICULUM VITAE

Personal Information	
Name:	**ALLYSSA MAE C. CANTOS**
Age:	18
Gender:	Female
Date of Birth:	April 22, 2005
Place of birth:	Rosario, Batangas
Current Address:	Magallanes, Luna, La Union
Civil Status:	Single
Nationality:	Filipino
Email:	Allyssamaecantos05@gmail.com

Educational Background
Elementary: Luna Central School Secondary: Regional Science High School for Region 1

CURRICULUM VITAE

Personal Information	
Name:	**KIM JEYEON A. DATO**
Age:	17
Gender:	Female
Date of Birth:	August 12, 2005
Place of birth:	San Fernando City, La Union
Current Address:	Rissing, Bangar, La Union
Civil Status:	Single
Nationality:	Filipino datokimjeyeon@gmail.com
Email:	

Educational Background
Elementary: Rissing Elementary School Secondary: Regional Science High School for Region 1

CURRICULUM VITAE

Personal Information	
Name:	**DAPHNE KATE V. VALDEZ**
Age:	18
Gender:	Female
Date of Birth:	August 14, 2005
Place of birth:	Balaoan, La Union
Current Address:	Corooy, Santol, La Union
Civil Status:	Single
Nationality:	Filipino valdezdaphnekate14@gmail.com
Email:	

Educational Background
Elementary: Corooy Elementary School Secondary: Regional Science High School for Region 1