ALOE VERA (*Aloe barbadensis*) AND HONEY (*Apis mellifera*) AS NATURAL FIXATIVES FOR TISSUE FIXATION IN HISTOPATHOLOGY

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Abstract

This study explores the potential of Aloe vera *(Aloe barbadensis)* and honey for use in tissue fixation for histopathology which has led researchers to explore safer, natural practices in tissue preservation. Aloe vera and honey are known for their preservative and antimicrobial properties, which may help maintain tissue structure and quality. This research examined how effective different ratios of Aloe vera and honey mixtures are as fixatives. Liver tissue from a pig was treated with various concentrations of these mixtures, and the resulting tissue quality was compared to samples preserved in 10% formalin. Histological factors like fixation quality, processing quality, tissue block integrity, sectioning quality, staining quality, and overall morphology were evaluated. Aloe vera and honey combinations showed preservation outcomes similar to formalin. Three formulations were tested: Treatment 1 (50% Aloe vera, 50% honey), Treatment 2 (75% Aloe vera, 25% honey), and Treatment 3 (25% Aloe vera, 75% honey). Treatment 3 (25% Aloe vera, 75% honey) showed fixation and processing quality comparable to formalin, with smoothest sectioning, suggesting honey aids cutting. Staining was consistent across all treatments. Treatment 3 also provided the most favorable tissue morphology, demonstrating its potential for preserving fine tissue details. These results support Aloe vera and honey mixtures as effective, less toxic fixatives in histopathology, promoting safer laboratory conditions and reduced environmental impact. Further studies are encouraged to confirm these results and assess their long-term effectiveness in tissue preservation.

***Keywords*:** *natural fixatives; aloe vera; honey; tissue preservation; histopathology*

Introduction

Fixation is one of the fundamental steps in tissue processing. Fixation as defined by Dey (2022), is the first step of any histological and cytological laboratory technique in tissue or cell processing. It is the process by which the cells in the tissue are fixed in a chemical and physical state, and all the biochemical and proteolytic activities within the cells are prevented so that the cells or tissues can resist any morphological change, distortion, or decomposition after subsequent treatment with various reagents. The fixation helps to maintain the tissue nearest to its original state in the living system. Its primary purpose is to preserve the tissue nearest to its living state; to prevent any change in shape and size of the tissue at the time of processing, autolysis, and any bacterial growth in the tissue; to make the tissue firm and hard and make it possible to have a clear stain; lastly, to have a better optical quality of the cells.

The quality of tissue fixation is crucial in histopathological analysis, as it directly influences the preservation of tissue architecture and cellular details. Formalin or formaldehyde is the most common fixative that is used in histopathology. Because of its consistent retention of tissue architecture, neutral buffered formalin is the most preferred fixative in formaldehyde for routine histopathological specimen fixation. It consists of formaldehyde which is buffered with sodium dihydrogen phosphate and disodium hydrogen phosphate at pH 6.8. This buffering system also inhibits the production of formalin pigment, which is the black precipitate formed due to acid hematin. To meet the various preservation requirements, many improved forms of formalin fixative have been designed. Calcium formalin containing calcium chloride is meant for the preservation of lipids, and phospholipids in particular. Formalin saline, a simple isotonic saline solution with formaldehyde, was historically popular before phosphate buffered formalin, but it often produces formalin pigment. Alcoholic formalin and formol acetic alcohol serve the dual purpose of providing the dehydrating effect of alcohol and the cross-linking effect of formaldehyde. Formol acetic alcohol has a faster action because of the presence of acetic acid and therefore it is ideal for making cryostat sections and promotion of tissue processing later. The pathologist, histologist, or researcher will have fully developed the understanding of the specific histological characteristics of tissues preserved with a given fixative and processed with a certain schedule or time (Rolls, 2024).

As formalin is known to have its harmful carcinogenic effects, it is according to the study of Pant and others in 2020, they cited that intense bodily fluid layer aggravation is the most widely recognized antagonistic impact of formaldehyde exposure, frequently prompting dry skin, dermatitis, tearing eyes, wheezing, and hacking. At levels of 25-50 parts per million, tissue harm might happen. At the same time, once exposure is removed, recovery tends to be rapid and complete. Aqueous solutions of formaldehyde are emphatically bothersome to the eye and may cause serious eye damage and injury. Formaldehyde can enter the body through inhalation, ingestion, or dermal retention. After absorption, it undergoes enzymatic oxidation primarily in the liver and to a lesser extent in erythrocytes, converting it into formic acid. Various reports have recorded the lethal impacts of formaldehyde exposure through several experimental studies since it is retained by all surfaces of the body.

When excessive formaldehyde enters the circulatory system, it is converted into formic acid, which can rapidly damage cells in the liver, kidneys, heart, and brain.

The principle of fixation in histopathology ensures that tissue structure is retained and diagnostic accuracy is ascertained. Fixation involves the utilization of chemical reagents to impede the autolytic process and therefore prevent putrefaction that may start setting in some minutes after death due to the presence of intrinsic enzymes and external organisms (Ajileye and Esan, 2022). This is paramount because it retains tissue architecture and its chemistry within a state more or less similar to life.

Formic acid can be eliminated through the kidney as sodium salt or further oxidized to carbon dioxide and water. Skin refinement following dermal exposure to formaldehyde has been very much archived. Human skin affectability factor by formaldehyde has been related with numerous circumstances of dermal exposure, contact with formalin, formaldehyde-containing pitches, formaldehyde-treated textures, formaldehyde-containing family items, facial tissues, and so forth. The progressively serious introduction brings about solidifying and tanning of the skin because of protein coagulation. Most cases of dermatitis are triggered by direct contact with formalin, although reactions from inhaling formaldehyde fumes have also been reported. People working in healthcare, dentistry, cosmetology, textile production, and construction are especially at risk due to frequent occupational exposure. (Pant, et al., 2020).

Ingesting a significant amount of formaldehyde can lead to irritation and burning of the mouth and throat, as well as damage and ulceration of the gastrointestinal tract. This may be accompanied by chest or abdominal pain, nausea, vomiting, diarrhea, and gastrointestinal bleeding. The intake of formalin also causes immediate inflammation of the linings of the mouth, throat, and digestive system. Furthermore, formaldehyde ingestion can result in metabolic acidosis, rapid breathing, jaundice, protein and blood in the urine, and acute kidney failure (Kim, et al., 2011).

Studies have shown that exposure to formaldehyde can cause damage to DNA and chromosomes in human peripheral blood cells. Evidence suggests that formaldehyde itself and not one of its metabolites can directly interact with DNA, leading to genotoxic effects in tissues at the entry point of exposure, especially when the body's capacity to metabolize the substance is overwhelmed (Baek, et al., 2018).

In a study conducted by Ahme and others in 2020, entitled “Toxic effects of formalin on the medical students of South Valley University following repeated exposure at the anatomy laboratories”, it statistically showed that approximately 79.6% of the 167 students suffered from unpleasant smell, followed by eye irritation (63.6%), nasal irritation (51.5%), headache (44.9%), breathing difficulties (39.5), visual disturbance (29.5), lack of concentration (26.9%), cough (25.1%), lethargy and fatigue (19.2%), nausea (18%), digestive disturbance (14.4%), sore throat and dryness (12%), sleep disturbance (4.8%) and fainting (6%). Students exhibited an awareness of the potential health risks associated with formalin exposure; however, they reportedly did not receive formal training or informational sessions regarding personal protective measures. Despite this lack of institutional guidance, a majority of students self-adopted

the use of laboratory coats and gloves as their primary means of protection during classes.

Formalin's popularity stems from its affordability, simplicity in preparation, and widespread, established use. It is often regarded as the best fixative, fostering what some have termed the "formalin dogma"—a firmly held belief that has, in some cases, limited exploration into alternative fixation methods (Gotur, S.P., et al 2023).

Despite extensive research, no single fixative has been able to completely replace formalin. While formalin is known to be hazardous and carcinogenic, particularly linked to nasopharyngeal cancer, efforts have been made to find natural compounds as alternatives for tissue fixation. Annually, a medium-large structure annually uses more than 3,500 liters of ready-to-use formalin (formaldehyde) as a general fixative in histopathology laboratoriesBoth honey and aloe vera have emerged as promising candidates for this role due to their unique chemical compositions and biological properties (Zanini et al., 2012).

Tissue samples should be immediately immersed in an appropriate fixative to ensure effective fixation. According to Ajileye and Esan, 2022, the volume of the fixative recommended is about 50 times that of the tissue. The fixative to be used depends on the type of tissue, as well as the specific histological features to be demonstrated, since different fixatives preserve morphology differently.

A good fixative should penetrate the tissue rapidly, prevent distortion during subsequent processing, and enhance visibility of cellular components for staining purposes. In the final analysis, proper fixation forms the bedrock of all histological preparations since it greatly impacts morphological interpretation and the reliability of histochemical or immunohistochemical analyses (Ajileye and Esan, 2022).

Aloe vera, often referred to as medicinal aloe, is a succulent species within the Aloe genus that is believed to have originated in Sudan. This plant thrives in arid climates and is found in various regions around the world. Aloe vera is recognized as a superior fixative for tissues in histopathology due to its high water content, polysaccharides, and other bioactive substances, which help maintain the integrity of fixed tissue. Aloe vera's composition, which includes over 75 active components such as polysaccharides, vitamins, and bioactive compounds, contributes to its effectiveness. These compounds provide antioxidant, immune-boosting, and tissue-preserving properties, making it a safer alternative to formalin, a known hazardous and carcinogenic fixative (Narwal et al., 2023).

In the study entitled "Qualitative Assessment of Aloe Vera as a Natural Tissue Fixative: An Institutional Study" by Narwal et al. (2023), Aloe vera was proven to be an effective natural fixative for preserving tissue morphology during histological preparation. The results highlighted that Aloe vera achieved fixation quality comparable to 10% formalin, which is the standard fixative in histopathology laboratories. The study showed that tissue shrinkage observed with Aloe vera was almost similar to formalin and less pronounced compared to other natural alternatives, such as sugar

solution and jaggery (Narwal et al., 2023). While past studies demonstrated aloe vera's potential as a formalin-comparable natural fixative for tissue morphology, this study explores an approach by investigating the combined effects of aloe vera and honey.

When tissues fixed in Aloe vera gel were stained with hematoxylin and eosin, they exhibited excellent nuclear and cytoplasmic details without tissue folding. This positioned Aloe vera as the second most effective fixative after formalin, outperforming other natural substances like honey and jaggery in maintaining tissue morphology and staining quality (Narwal et al., 2023).

The authors proposed that the fixative action of Aloe vera could be attributed to active components in its gel, such as aloemannan and acemannan, which under acidic conditions convert to aldehyde-like compounds. These compounds mimic the fixation properties of formaldehyde, ensuring uniform and thorough tissue preservation. Furthermore, Aloe vera's antibacterial and antifungal properties add to its efficacy by preventing tissue decomposition during the fixation process (Narwal et al., 2023). As a well-known herbal medicine, Aloe vera exhibits potent antibacterial properties and contains compounds that protect living cells, promoting its use in laboratory settings. (Smith, J., & Doe, P., 2023)

The study by Narwal et al. (2023) concluded that Aloe vera gel has significant potential as a natural fixative, particularly in situations where formalin is unavailable or unsuitable. While its application in routine hematoxylin and eosin staining has yielded promising results, future studies are recommended to explore its effectiveness in histochemical and immunohistochemical staining methods. By serving as a non-hazardous and eco-friendly fixative, Aloe vera could advance histopathological practices and address safety concerns associated with formalin (Narwal et al., 2023).

Studies have demonstrated that distortions in the microscopic structure of tissues fixed with Aloe vera are minimal, with both axial and cytoplasmic details preserved at levels comparable to those achieved with formalin fixation. (Sabarinath et al, 2024). Additionally, Aloe vera-fixed specimens yield highly Fair results with hematoxylin and eosin (H&E) and other staining methods, suggesting that Aloe vera is a safe, eco-friendly option well-suited for routine histopathological procedures. (Singh, et al., 2015).

Honey, another natural substance, is produced by honey bees when they collect, modify, and store nectar and sweet secretions from plants in their honeycomb. Like Aloe vera, honey is also recognized as a natural fixative in histopathology. The biochemical composition of honey, including its high sugar content, low water activity, acidic pH, and enzymatic production of hydrogen peroxide, contributed to its fixative properties. These factors help to inhibit microbial growth while facilitating tissue preservation. Processed honey, which has a higher pH (5.05), proved to be more effective in preserving tissue morphology than unprocessed honey (pH 3.6), which exhibited more artifacts, such as tissue shrinkage and compromised cellular structure (Lalwani et al., 2015).

While Aloe vera has shown promising results as a natural fixative, offering comparable preservation of tissue morphology to formalin and outperforming other alternatives like jaggery, honey also presents an intriguing natural alternative. In the study "Honey as an Alternative Fixative for Oral Tissue: An Evaluation of Processed and Unprocessed Honey" by Lalwani et al. (2015), honey was evaluated for its potential as a tissue fixative, revealing that it offers a safer and effective substitute for formalin in preserving tissue architecture.

The results of the study demonstrated that both processed and unprocessed honey were highly effective at preserving tissue morphology and were able to provide 100% efficiency in nuclear staining, comparable to neutral buffered formalin (NBF). Cytoplasmic staining for honey-treated samples was also found to be 92% effective, which was similar to neutral buffered formalin. However, tissue morphology adequacy was slightly lower at 75% for honey al., 2015).

Its composition, primarily consisting of fructose and glucose, along with some organic acids and very low concentrations of hydrogen peroxide, creates an unwelcoming environment for microbes while effectively liquefying tissues (Alwahaibi, et al., 2023). This antimicrobial property enables honey to maintain cell and tissue architecture, which is essential for histological assessment. The low pH of honey aids in tissue preservation and provides a hardening effect, allowing for the prolonged preservation of cells (Singhal, P., et al., 2017).

Moreover, honey-fixed tissues demonstrate good compatibility with H&E and other histology stains, making it suitable for standard histopathological protocols. Its lower toxicity compared to formalin significantly reduces health risks to laboratory personnel and aligns with eco-friendly practices. Honey also provides clarity comparable to formalin, which is crucial for accurate diagnostic procedures. Additionally, honey's acidic pH inhibits autolysis, leading to stabilized cells and preserved tissue structure (Singhal, P., et al., 2017). Its compatibility with commonly used stains, such as H&E, allows for seamless integration into standard histological workflows, ensuring minimal color interference during detailed staining processes (Sabarinath et al., 2024).

Formaldehyde is widely recognized in histological practices as an effective fixative solution, valued for its ability to preserve cellular structures for microscopic analysis. However, the toxic and carcinogenic properties of formaldehyde raise substantial health and environmental concerns, highlighting the need for safer, non-toxic alternatives. This study evaluates the effectiveness of Aloe vera (Aloe barbadensis) and honey as natural fixative solutions, investigating their ability to maintain tissue integrity at levels comparable to those achieved with formaldehyde. Both Aloe vera and honey possess well-established preservative properties suggesting their potential as effective substitutes that reduce health risks to laboratory personnel and mitigate environmental impact while preserving tissue morphology for histological assessment.

According to Lada and others in their study, “Porcine Liver Anatomy Applied to Biomedicine” in 2020, the domestic pig liver is suitable for preclinical research because

of its close anatomical, physiological, and dimensional similarities to the human liver; in addition, there is a high degree of genetic similarity between the two species. It is suitable for tissue processing because according to a study of Tarbet and others of Leica Biosystems, it contains hepatocytes with characteristic morphological features in association with delicate sinusoidal vessels, and also present is connective tissue containing vessels and ducts. In the pork liver – unlike the liver of other animals intended for human consumption – the connective tissue is visible because it is surrounding its lobules, forming a fibrous envelope that interconnects the adjacent portal areas. In addition, it can also be found within lobules, both adjacent to the sinusoids and around the central veins (Mik et al., 2018).

Honey and aloe vera are known to be local products in the Province of La Union. Hence, the utilization of these products can also support the locals to boost their economies and income. By using these natural products, we can reduce the costs compared to when using formaldehyde as a fixative solution. Also, it is less toxic to laboratory personnel which makes it safer for them and has a lower environmental impact. Overall, the study aimed to replace formalin to a much safer alternative and to have a more cost-effective solution.

This research concerning aloe vera (Aloe barbadensis) and honey (Apis millifera) as a supplementary option to formaldehyde as a fixative solution aims to determine the effectiveness of the solution in preparing histology specimens. This study explores the effectiveness of different ratios of aloe vera, honey solution, against formaldehyde in staining quality, cellular outline, nuclear details, and overall morphology under light microscopy. This study aimed to investigate the cost-effectiveness and safety of aloe vera and honey as a supplementary option to formaldehyde in tissue specimen preparation. The research aimed to assess the most effective method for tissue specimen preparation.

The study investigating Aloe vera (Aloe barbadensis) and honey as supplementary options to formaldehyde presents a promising shift in tissue fixation for medical and research applications. The significance of this study lies in its potential to provide safer, more sustainable alternatives to formaldehyde, a widely used but toxic fixative. Aloe vera's polysaccharides, including aloemannan and acemannan, have been shown to replicate formaldehyde's cross-linking effects, while honey contributes to preventing tissue decay due to its hygroscopic, antimicrobial, and antioxidant properties. Both of these natural products are non-toxic, biodegradable, and come from renewable resources, making them not only safer but also environmentally sustainable alternatives. As research progresses, these findings could lead to refined formulations of Aloe vera and honey that would enhance their effectiveness and increase their use in various laboratory environments (Badakhsh et al., 2023).

The complementary action of Aloe and honey in tissue preservation does not involve the injurious consequences of formaldehyde such as the risk of cancer. Hence, these agents guarantee excellent histological specimens through uniformity in stain absorption and structure stabilization. These mechanisms will inhibit bacterial proliferation and lysis, which would be detrimental to the preservation of the tissues.

These fixatives, therefore, become valuable where protection from formaldehyde is a serious concern or in resource-poor settings where economical substitutes are needed (Tserenbat et al., 2023).

The implications of this study are probably the beginning of revolutionary changes to occur in the production of safer alternatives for fixatives. Aloe vera (Aloe barbadensis) and honey with the global-warming friendly, health-friendly will assist in promoting more widespread use of non-toxic alternate chemicals to formaldehyde. This work benefits the histology and pathology domains while also acting as an engine for promoting safe and sustainable laboratory practice. As time goes on, this research has the potential to set a new normative standard for health and environmental safety within the medical fold, thereby creating an avenue for safer and greener tissue preservation approaches.

Objectives

General Objective:

To evaluate the effectiveness of Aloe vera (*Aloe barbadensis*) and honey mixtures as natural fixatives for tissue fixation in histopathology compared to a standard fixative, formalin.

Specific Objectives:

To determine the degree of effectiveness of different treatments of Aloe vera and honey mixtures in fixing pig liver tissue specimens based on fixation quality.

To assess the degree of effectiveness of different treatments of the Aloe vera and honey mixture as a natural tissue fixative by evaluating tissue processing parameters, including processing quality, tissue block integrity, sectioning quality, staining quality, and overall morphology.

To identify if there is a significant difference in the effectiveness of the Aloe vera and honey mixture as a natural tissue fixative compared to the control, formalin.

Materials and methods

Research Design

This study used a quantitative experimental research design to assess the effectiveness of aloe vera (Aloe barbadensis) and honey as fixative solutions in the preparation of tissue samples. According to Zubair (2022), it is a scientific way of doing research in which one or more independent variables are modified and applied to one or more dependent variables to determine their impact on the latter. This assisted the researchers in manipulating the independent variable in the study, which is the process of using different ratios of aloe vera and honey solution as the fixative, whereas the dependent variables are the outcomes of the staining quality, cellular outline, nuclear details, and overall morphology of the histology specimen under light microscopy. The

histological properties were evaluated following the application of various fixative solution ratios.

This study used an experimental research design to look into the effectiveness of aloe vera and honey as a fixative solution for processing tissue samples. The numerical data acquired was statistically examined to determine the most effective ratio to utilize, as well as whether there are significant differences between treatments and how well this alternative works in preserving tissue specimens.

Specimen and Locale of the Study

The aloe vera leaves were collected from Agoo, La Union to ensure the right environment and to minimize the alterations due to chemicals and fertilizers to also keep the freshness of the material at its highest quality to avoid differences throughout the experiment.

Honey was acquired from the Western honey bee (Apis mellifera) beekeepers at the Don Mariano Marcos Memorial State University-North La Union Campus, supporting the local agricultural economy which is known for their honey bee fields while ensuring the use of fresh, unadulterated honey. Bacnotan is the seat of the beekeeping industry in the province of La Union, and is the home of the Apiculture Center of the Philippines as they have the training and facility to help people grow bees, giving consumers the best quality of pure and concentrated honey.

The liver of pigs (Sus domesticus), was locally sourced at the City Slaughterhouse at Brgy. Tanqui, City of San Fernando, La Union. The quality of the liver they sold is high quality and suitable for the conduct of the study because it is a facility for butchering pigs with hygienic and clean procedures providing that the pig liver we sourced is not contaminated. Since these materials are sourced locally, the study minimizes transportation problems and guarantees the freshness and reliability of the specimens.

To uphold animal ethical standards in data collection, the specimens collected were treated with respect and dignity, regardless of their utility value. The researchers counterbalanced the harm with the benefit of conducting the research. The primary focus of the study was the risk-to-benefit ratio. If the risks had exceeded the possible benefits, the study would have been discontinued or limited. The researchers acknowledged the outcome of their experiment.

The evaluation committee was composed of five qualified evaluators, all of whom were registered medical laboratory technologists with the Faculty of the College of Medical Laboratory Science. The committee conducted a comprehensive assessment of each submitted specimen, systematically evaluating several critical parameters. These parameters included fixation quality, processing quality, sectioning quality, staining quality, tissue block integrity, and the overall morphological presentation of the tissue samples.

Data Gathering Tool

The study employed a combination of tools and methodologies to ensure accurate and systematic evaluation of tissue samples treated with different fixative solutions. Microscopic observation using a light microscope, Olympus Cx23, served as the primary method for assessing the effectiveness of the fixative solutions. Observations focused on six key parameters: fixation quality, processing quality, tissue block integrity, sectioning quality, staining quality, and overall morphology under light microscopy.

These parameters were essential for determining how well the fixatives preserved the structural and morphological integrity of the tissue samples.

Data Gathering Procedure

Consent

The researchers obtained permission from the College of Medical Laboratory Science RTP and the Lorma Colleges Research Ethics Committee. The consent form was reviewed and approved by the researchers' teacher and research adviser, who were both registered medical technologists. Following this, the researchers obtained consent from the evaluators who were faculty members of the College of Medical Laboratory Science and practicing medical technologists, all registered. Prior to obtaining consent, the researchers provided a detailed explanation of the study procedures, emphasizing that participation was entirely voluntary and that participants had the right to withdraw at any point without penalty or negative consequences. The researchers implemented confidentiality measures to ensure the privacy of the evaluators' identities and responses. They were also informed of how the data would be utilized, stored, and eventually disposed of to uphold ethical data management practices. To confirm their understanding and willingness to participate, evaluators signed the consent form, which served as a formal agreement indicating their informed and voluntary participation. This process was designed to maintain transparency, build trust, and uphold the ethical standards of research within the field of medical technology. Once the consent form was approved, the researchers discussed the process of the study, participation, possible unfavorable factors, and the benefits of the study with the evaluators.

Materials and Reagent Use

For the collection of aloe vera, beaker glassware, sieving/filtering gauze, muslin cloth, knife, chopping board, and basin were used. The glassware used was thoroughly washed with water and then autoclaved to avoid any microbial or chemical contamination that might affect the analysis. Type III water was used for cleaning the dirt residues from the aloe vera plant. For the specimen preparation and collection, kitchen wares like knife, chopping board, basin, NSS and digital analytical weighing balance were used. For the specimen transportation, four sterilized jars were used for containing the specimens right after the collection of the pig's liver.

The laboratory equipment used for the assay includes a sterile knife and a chopping board for tissue processing, Four 1,000 mL airtight glass jars, two 500 mL beakers, four stirring rods for solution preparation, eight 500 mL were used for infiltration, twelve glass staining jars were used for dehydration, clearing and staining. This comprehensive collection and preparation process ensured the quality and reliability of the fixative solutions and tissue samples for the study. For the tissue processing, a paraffin oven, laboratory stove, histological cassette, rotary microtome, water bath, glass slides, and paraffin wax were used. Alcohol solutions (70%, 90%, and 100%) were also used for dehydration and xylene as the clearing agent for the clearing process. Reagents such as hematoxylin, eosin, acid alcohol, ethanol,and xylene were used for staining. A mounting media was used to preserve the slide and for the visualization of the quality of the stained specimen, a light microscope (Olympus Cx23) was used and an immersion oil for OIO viewing.

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Collection of Honey and Aloe Vera

The sourced materials, honey and aloe vera was sent to the College of Agroforestry and Forestry of the Don Mariano Marcos Memorial State University - North La Union Campus in Bacnotan, La Union for proper identification, species authentication and verification of the raw materials, including the Aloe vera leaves and honey.

The materials for the preparation of the fixative solutions include approximately 6 kg of mature Aloe vera leaves. The leaves were harvested early in the morning to ensure maximum moisture retention. The harvested leaves were washed thoroughly with distilled water to remove dirt and impurities, then allowed to air dry before use.

Honey was sourced from Western honey bee (Apis mellifera) beehives in which the beekeepers extracted the honey directly from the combs using traditional honey extraction techniques to preserve its natural properties and was packaged in a sterilized container. The purchased honey was then transported to the Medical Laboratory Science Laboratory in Lorma Colleges for further processing.

Aloe Vera Solution Extraction and Preparation

Approximately 6 kilograms of Aloe vera (Aloe barbadensis) leaves were used for extraction of approximately 1300 mL of aloe vera. The outer surface of the aloe vera leaves were washed with type III water and disinfected with 70% ethanol. The gelatinous transparent substance was cut off from the green rind or the cuticle of the aloe vera leaves and blended using a blender to get a homogeneous gel. The homogeneous gel was strained using a sterile sieving gauze to extract the aloe vera juice, followed by another filtration using another clean sieving gauze to obtain a liquid extract separated from the solid plant material. The volume needed for the solution was then measured using a beaker.

Honey Solution Preparation

The containers of the honey were already sterilized upon purchasing. Before opening, the honey was sterilized by passing the lid portion of the container over the flame of the alcohol lamp in a circular motion to avoid microbial contaminations, and then cooled in a cooling rack. The desired volume of honey to be used was then measured using a beaker.

Preparation of Fixative Solution for Liver Specimen Transportation

The preservative solution mixtures were prepared in 1000 mL in four sterilized air tight jars. For the first preservative solution (first jar), it was prepared by mixing 250 mL of aloe vera solution and 250 mL of honey solution. The second jar contained 375 mL of aloe vera solution and 125 mL of honey solution. For the third jar, it contained 125 mL of aloe vera solution and 375 mL of honey solution. The fourth jar contained the positive control, 500 mL of 10% buffered formalin. Each preservative solution in the jars followed the concentration ratios of the treatment samples: 50%(aloe vera solution):50%(honey solution), 75%(aloe vera solution):25%(honey solution), 25%(aloe vera solution):75%(honey solution) and the positive control solution (10% formalin solution).

Liver Tissue Specimen Preparation

To ensure the definite species used, the pig liver was identified at the City Slaughterhouse at Brgy. Tanqui, City of San Fernando, La Union by the City Veterinarian.

The liver of a pig (*Sus domesticus*) weighing 500 grams, was bought at the slaughterhouse of the City of San Fernando. The liver specimen was portioned into 50 grams for each treatment and washed with normal saline solution (NSS). Each 50 grams of liver were then placed in four sterilized jars with the respective solutions: 250 mL aloe vera solution and 250 mL honey solution, 375 mL aloe vera solution and 125 mL honey solution, 125 mL aloe vera solution and 375 mL honey solution, and 500 mL 10% buffered formalin. Upon arrival at the laboratory after approximately 30 to 45 minutes of transport, each liver was visually inspected for any defects such as discoloration and observable build up of molds and maggots– no defects were found and proceeded with the cutting.

Preparation of Treatment Samples

The fixative solution mixture was prepared in a 1000 ml jar. Treatment 1 was prepared by mixing 50 mL of aloe vera solution and 50 ml of honey solution. For treatment 2, 75 mL of aloe vera solution were mixed with 25 mL of honey solution. Treatment 3 was prepared by mixing 25 mL of aloe vera solution with 75 mL of honey solution. The tissues were also fixed using a positive control. 100 mL of 10% buffered

formalin was poured also in a separate jar for positive control. Each treatment has three replicates. The different treatments are presented in Table A.

Table A Treatment Distribution

Treatments (Three replicates)	Ratio	Constituents
T+		100 mL of 10% Buffered formalin
T1	(50%:50%)	50 mL of aloe vera solution and 50 mL of honey solution
T2	(75%:25%)	75 mL of aloe vera solution and 25 mL of honey solution
T3	(25%:75%)	25 mL of aloe vera solution and 75 mL of honey solution

Tissue Processing

Labelling

Each histological cassette was labelled corresponding to their treatments and group replicates . For the control treatment (T+) and its replicates, the cassettes were labeled as, “H+001, H+002, and H+003”. The labels “H1001, H1002, and H1003” was used for treatment 1 (T1) and its succeeding replicates. For treatment 2 (T2) and its replicates, the cassettes were labeled as “H2001, H2002, and H2003”. Lastly, “H3001, H3002, and H3003” were used to label the cassettes for treatment 3 (T3) and its replicates. The labels assigned for each treatment were then used for the tissue processing procedures.

Fixation

Each tissue specimen was cut into 4 mm × 4 mm × 4 mm and placed in histological cassettes. The cassettes were immersed and were fixed in its respective treatment solution at room temperature for 24 hours to penetrate the tissue causing chemical and physical changes that hardened and preserved the tissue and protected it against subsequent processing steps. After fixation, the difference between pre-and post-fixation measurements of each specimen was recorded to assess tissue shrinkage or expansion.

Dehydration

Following this, the tissues underwent dehydration by immersion in a graded series of ethanol solutions (70%, 90%, and 100%), within their respective timeframe to avoid excessive distortion of tissue until a water-free tissue in alcohol is reached. The tissues were dehydrated in 70%, 90% and 100% ethanol for 15 minutes each and two changes of 100% ethanol for 45 minutes each. Ethanol is miscible with water in all proportions so that the water in the specimen is progressively replaced by the alcohol. This step ensures the removal of water and prepares the tissues for subsequent clearing.

Clearing

The dehydrated tissues were then immersed in three different xylene immersions for the clearing process. The tissues were submerged for 45 minutes each for the first and second immersions. This procedure replaces the ethanol with a reagent compatible

with the embedding medium since xylene is miscible with both ethanol and paraffin wax and removes fat from the liver tissue which can present a barrier to wax infiltration.

Infiltration

After clearing, the tissues were infiltrated with paraffin wax, which has a melting point of 56-58°C. For the first and second impregnation, the tissues underwent impregnation for 30 minutes each and 45 minutes for the last impregnation. In this process, the clearing agent was completely removed from the tissue and replaced by a medium that completely filled all the tissue cavities, thereby giving a firm consistency to the specimen, and allowed easier handling and cutting of suitably thin sections without any damage or distortion to the tissue and its cellular components.

Embedding

The paraffin-infiltrated tissue were placed in a paper boat with melted paraffin and were allowed to harden to produce solid tissue blocks to be later clamped into a microtome for sectioning. The tissues were oriented in the mold to prevent important tissue elements being missed or damaged during microtomy, and then filled with melted paraffin wax. The whole mold was placed on a cold plate to solidify. After the cooling of the paraffin, the block was removed from the mold and proceeded to sectioning on a microtome. This step allowed the hardening of tissues, giving them a firmer consistency and better support, thereby facilitating the cutting of sections.

Sectioning-Cutting (Microtomy)

Using a rotary microtome, thin sections measuring 4 μm were cut from the paraffin blocks to allow great visual representation and to avoid sections curling in on themselves.

Mounting of Tissue Sections

Serial paraffin ribbons of tissue sections were taken out of the microtome with an applicator stick to apply a gentle pull on the ribbon end. The tissue was stretched slowly over the applicator stick while floating in a warm bath of water for no more than 1 or 2 minutes to prevent excessive tissue hydration. The water bath was maintained at 37-38oC to prevent undue wrinkling of the tissue or desiccated appearance of the sections. Albumin was utilized as a cementing agent and was added to the glass slide for the tissue specimen to attach to the glass slides. The tissue sections, after mounting, were allowed to dry for around 15 minutes to secure the proper attachment to the slides. Staining

Staining

Finally, the prepared slides were stained with hematoxylin and eosin (H&E) to highlight cellular structures and morphological features. The slides were treated with 95% ethanol for 3-5 minutes, stained with Harris hematoxylin for 10 minutes, and the stained slides were washed with water. The slides were dipped in 1% acid alcohol three times to selectively remove excess hematoxylin from non-nuclear areas of the tissue. The slides were dipped 7 times in eosin y alcohol for counterstaining and then rinsed off with water. After counterstaining, it was dehydrated in 95% alcohol for 1 minute and then cleared with xylene for 3 minutes. This step facilitates proper adhesion of the

mounting medium and minimizes artifacts that lead to difficulty in visualizing the specimen .

Interpretation of results

The stained tissue slides were examined under the microscope to assess the performance of the fixative solutions containing Aloe vera, honey, and formaldehyde. The criteria included fixation quality, processing quality, tissue block integrity, sectioning quality, staining quality, and general morphology and were inspected under light microscopy to find how well the structures of the tissue are maintained by the fixatives. Five competent observers, registered as medical technologists, independently scored the slides using a standard scoring system to ensure uniformity and eliminate subjective bias.

The tissue samples were analyzed and compared with the following criteria: (a) fixation quality, (b) processing quality, (c) tissue block integrity, (d) sectioning quality, (e) staining quality, (f) overall morphology under light microscopy.

Every parameter—quality of fixation, quality of processing, integrity of tissue blocks, quality of sectioning, quality of staining, and morphology overall—were graded from 1 (Poor) through to 4 (Excellent). The grades were determined using the clarity of stain, the cellular and nuclear structure definition, and morphological preservation of tissue, according to the grading criteria set out in Bancroft's Theory and Practice of Histological Techniques (Bancroft & Layton, 2019). A uniform grading system was used to provide consistency and reliability in the assessment process. Every parameter was graded on a scale of 1 to 4, in which 1 represents poor preservation, 2 represents Fair preservation with defects, 3 represents good preservation with slight imperfections, and 4 represents excellent preservation with no visible defects, i.e., tissue that is well-preserved, with well-preserved cellular structure, good processing, and even staining. Independent assessments were performed by registered medical technologists to reduce bias and increase the reliability of the results. Photo documentation complemented the assessment process. Images of the tissue sections at high resolution were taken during microscopic examination to serve as visual proof of the quality of the tissue. The images complemented the numerical ratings, enabling a close examination of the tissue characteristics. They also act as a reference for verification, checking the quality of the results and allowing further investigation as needed. All data collected were rigorously tabulated in tables that contained the rating for every parameter and respective photograph documentation. This organized presentation allowed for straightforward comparison of the various treatments and controls, readily displaying what each fixative solution performed. The revised grading matrix is shown on tables B, C, D, E, F and G.

To compare the data, the scores for every parameter—fixation quality, processing quality, tissue block integrity, sectioning quality, staining quality, and overall morphology.—were compared between all treatments. The 1–4 scoring system guarantees that the assessment is consistent and quantifiable. The data were reported in table format with scores for each parameter across the treatment groups. Mean scores for each parameter were determined for each treatment to give a general measure of

effectiveness. Numerical scores obtained from photographic documentation during microscopic observation were utilized to confirm the scores. Observers consulted the images to confirm that the assigned scores are consistent with the visual evidence. This process is used to address possible discrepancies and improve the accuracy of the evaluation. Comparisons were drawn between the various fixative treatments, and between the treatments and the positive (10% buffered formalin). Patterns and differences in preservation quality were discerned and discussed, with specific emphasis placed on the efficacy of Aloe vera and honey-based fixatives in comparison to the formaldehyde standard. Lastly, the findings were explained within the scope of the research aimed to ascertain the best fixative ratio in tissue sample preservation. The process of evaluation makes sure that both qualitative (photographic evidence) and quantitative (scores) information are incorporated so that an integrated picture of how well the fixative solutions performed is given. This systematic procedure makes sure that the findings are relevant, reproducible, and scientifically sound.

Table B Grading Criteria for the Fixation Quality of the Tissue Specimen

Grading	Statistical range	Descriptive equivalent
4 (Excellent)	3.26 - 4.00	Tissue is fully fixed, showing no autolysis, shrinkage, or hardening. Nuclear membranes and chromatin are crisp, and cytoplasmic details are intact.
3 (Good)	2.51 – 3.25	Tissue is mostly fixed but may have minor areas of uneven penetration or slight autolysis. Shrinkage is minimal.
2 (Fair)	1.76 – 2.50	Fixation is inconsistent, with areas of autolysis, moderate shrinkage, or improper penetration. Chromatin is less distinct.
1 (Poor)	1.00 - 1.75	Fixation is poor, with widespread autolysis, shrinkage, or incomplete penetration. Cellular details are lost.

Table C Grading Criteria for the Processing Quality of the Tissue Specimen

Grading	Statistical range	Descriptive equivalent
4 (Excellent)	3.26 - 4.00	The tissue has been processed very well. It shows proper dehydration and clearing, without any signs of remaining moisture or clearing agent.
3 (Good)	2.51 – 3.25	The tissue processing is generally good. There might be some small areas where dehydration or clearing isn't consistent, but it's not enough to cause significant problems.
2 (Fair)	1.76 – 2.50	The tissue processing shows noticeable inconsistencies. This has led to some soft spots within the tissue block.
1 (Poor)	1.00 - 1.75	The tissue processing was inadequate. This has resulted in uneven clearing, retained moisture, or insufficient paraffin infiltration.

Table D Grading Criteria for the Tissue Block Integrity

Grading	Statistical range	Descriptive equivalent
4 (Excellent)	3.26 - 4.00	Block is firm, smooth, and free of cracks or crumbling, ensuring excellent stability and usability.
3 (Good)	2.51 – 3.25	Block has minor surface cracks or slightly uneven texture but remains stable for sectioning
2 (Fair)	1.76 – 2.50	Block shows visible cracks, brittleness, or crumbling, affecting handling.
1 (Poor)	1.00 - 1.75	Block is severely compromised, with extensive cracking, crumbling, or instability that renders it unusable

Table E Grading Criteria for the Sectioning Quality of the Tissue Specimen

Grading	Statistical range	Descriptive equivalent
4 (Excellent)	3.26 - 4.00	Sections are even, thin, and free from tears, folds, or compression artifacts. No irregularities are visible under the microscope.
3 (Good)	2.51 – 3.25	Sections are mostly smooth, with slight tears or compression artifacts that do not obscure critical tissue details.
2 (Fair)	1.76 – 2.50	Sections show defects, such as tearing, folding, or compression, that partially obscure tissue details.
1 (Poor)	1.00 - 1.75	Sections are unusable, with extensive folds, tears, or compression artifacts obscuring tissue details entirely.

Table F Grading Criteria for the Staining Quality of the Tissue Specimen

Grading	Statistical range	Descriptive equivalent
4 (Excellent)	3.26 - 4.00	Staining is uniform, with excellent differentiation between nuclei and cytoplasm. No blotches or faded areas are observed.
3 (Good)	2.51 – 3.25	Staining is mostly uniform, with minor imperfections like faint areas or slight uneven application of dyes.
2 (Fair)	1.76 – 2.50	Staining is irregular, with visible artifacts, weak nuclear-cytoplasmic contrast, or incomplete coverage in some areas.
1 (Poor)	1.00 - 1.75	Staining is inadequate, with unstained areas, excessive blotching, or over-saturation that obscures cellular structures.

Table G Grading Criteria for the Overall Morphology of the Tissue Specimen Under Light Microscopy

Grading	Statistical range	Descriptive equivalent
4 (Excellent)	3.26 - 4.00	Tissue is fully intact, showing no breaks, gaps, or distortions. All structural components are preserved and clearly visible.
3 (Good)	2.51 – 3.25	Tissue is mostly intact, with minor tears, gaps, or slightly misaligned layers that do not hinder analysis.
2 (Fair)	1.76 – 2.50	Tissue shows separation of layers or partial loss of structural integrity, affecting visualization.
1 (Poor)	1.00 - 1.75	Tissue is severely damaged, distorted, or fragmented, making reliable analysis impossible.

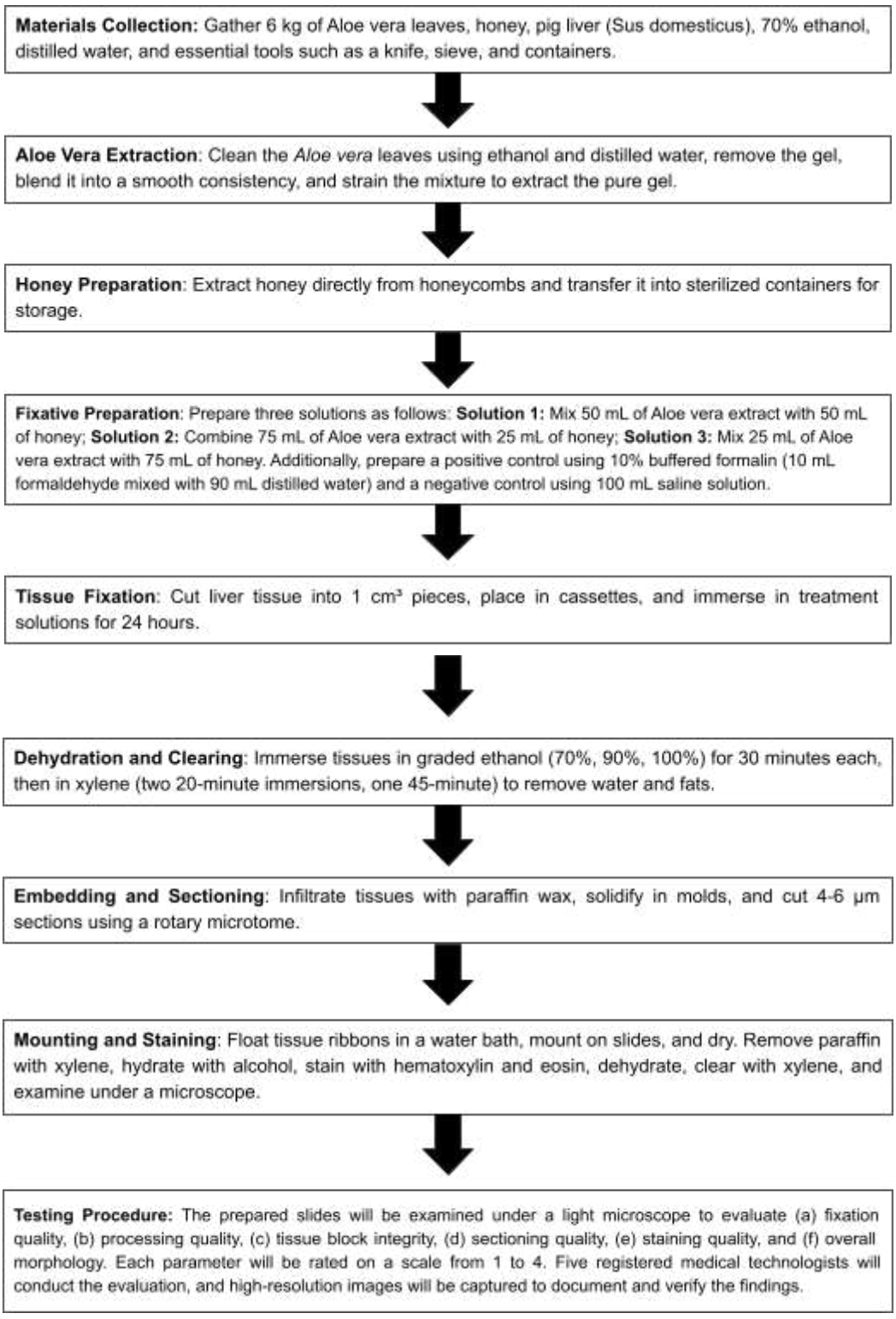

FIGURE 1: Summary of Data Gathering Procedure: Preparation of Aloe Vera and Honey Fixative Solutions, Tissue Fixation, Processing, and Histological Evaluation

Treatment of data

ANOVA was utilized in this study to determine whether statistically significant variations in the performance of Aloe Vera and Honey as fixative solutions exist in the various treatment groups. ANOVA is a good choice since it will allow comparing several treatment groups (with different Aloe Vera and Honey concentrations) simultaneously, to check whether the variations in tissue preservation observed are caused by the treatments or by chance. It is a good method to compare the mean values of the treatments to check whether one of them is significantly different.

Following ANOVA, Tukey's HSD (Honestly Significant Difference) Post-Hoc Test was used. This test identified which particular treatments are different from one another. Although ANOVA showed that there is a difference, it does not tell us which treatments are significantly different from one another. Tukey's test enabled us to make a more precise comparison, determining which combinations of Aloe Vera and Honey concentrations had the most pronounced effects on tissue preservation.

In summary of the data, descriptive statistics (mean ± standard deviation) were employed to describe the central tendency and variation of the outcomes in each treatment group. This gives a good picture of the efficacy of the fixative solutions among the different experimental groups.

The level of significance was at $p < 0.05$, such that any differences found in the results are not likely to have happened by chance and are statistically significant, hence verifying the efficacy of the treatments under test.

Ethical Consideration

During the conduct of this research study, there were various ethical considerations that were observed carefully to ensure institutional standards, ensure safety, and maintain the integrity of the research process. Before any procedures were done, permits and approvals had been obtained. Ethical approval was received from the Lorma Colleges Research Ethics Committee, confirming that the study conformed to laid down ethical standards. Sanitary permit approval was received from the City of San Fernando Slaughterhouse, where part of the study was carried out. Prior to submission of these documents for approval, all research-related papers were read and appropriately signed by the Dean of College of Medical Laboratory Science, the Research Coordinator, the Research Instructor, and the Research Adviser. Throughout the experimental procedures, laboratory safety procedures were strictly followed. Proper personal protective equipment such as lab gowns, gloves, and hairnets was worn by all researchers to ensure a sterile working area and to avoid contamination. The research did not include any human subjects, thus avoiding any ethical issues of consent or human subject involvement. All information collected during the research was treated with confidentiality and stored securely to maintain their accuracy and reliability. The research was fully self-funded, thus avoiding any possible conflict of interest and ensuring the objectivity of the findings.

Results

Table 1. Fixation Quality of the Different Treatments of Aloe Vera and Honey Solution

Treatment	Total Mean	Interpretation
T+	3.667	Excellent
T1	1.667	Poor
T2	2.667	Good
T3	3.533	Excellent

Table 1 indicates that Treatment 3 (25% Aloe vera, 75% honey) demonstrated excellent fixation quality with a total mean score of 3.533, comparable to the positive control (10% neutral buffered formalin) which scored 3.667. In contrast, Treatment 1 (50% Aloe vera, 50% honey) showed poor fixation quality with the lowest total mean score of 1.667, and Treatment 2 (75% Aloe vera, 25% honey) was rated as good with a mean score of 2.667. These results suggest that a higher concentration of honey in the mixture is more effective for tissue fixation, performing similarly to the standard formalin fixative.

Table 2. Degree Of Effectiveness Of The Aloe Vera And Honey Mixture In Different Treatments As A Natural Tissue Fixative

	Fixation Quality	Processing Quality	Tissue Block Integrity	Sectioning Quality	Staining Quality	Overall Morphology
Total Mean of T + (Interpretation)	3.667 (Excellent)	3.667 (Excellent)	3.667 (Excellent)	2.867 (Good)	2.200 (Fair)	2.067 (Fair)
Total Mean of T 1 (Interpretation)	1.667 (Poor)	2.733	3.667 (Excellent)	2.333 (Fair)	2.600 (Good)	2.467 (Fair)
Total Mean of T 2 (Interpretation)	2.667 (Good)	2.667	3.667 (Excellent)	2.400 (Fair)	2.467 (Fair)	2.467 (Fair)
Total Mean of T 3 (Interpretation)	3.533 (Excellent)	3.533	3.667 (Excellent)	2.800 (Good)	2.600 (Good)	2.800 (Good)

Table 2 presents the effectiveness of the different treatments across several tissue processing parameters. All treatments, including the positive control (T+), Treatment 1 (T1), Treatment 2 (T2), and Treatment 3 (T3), showed excellent tissue block integrity with a total mean score of 3.667. For processing quality, both T+ and T3 were rated as excellent with total mean scores of 3.667 and 3.533 respectively, while T1 and T2 were rated as good. T+ showed the highest sectioning quality (2.867), closely followed by T3 (2.800), both rated as good, whereas T1 and T2 were rated as fair. Staining quality was rated as fair for T+ and T2, and good for T1 and T3. Finally, for overall morphology, T+ and T1 and T2 were rated as fair, while T3 showed good overall morphology with a mean score of 2.800. These findings collectively indicate that Treatment 3 with a higher honey concentration consistently performed well across most processing parameters, often comparable to the formalin control.

Table 3. Significant Difference in the Effectiveness of Aloe vera and Honey Mixture as a Natural Tissue Fixative and the Control for Tissue Fixation in Histopathology Across Fixation Quality

Source	*SS*	*df*	*MS*	*F*	*p-value*
Treatment	13.290	3	4.4300	28.89	.0001
Error	1.227	8	0.1533		
Total	14.517	11			

Table 3.1. Post hoc analysis using p-values for pairwise t-test

p-values for pairwise t-tests		**T2**	**T1**	**T+**	**T3**
		1.47	**1.67**	**3.67**	**3.67**
T2	1.47				
T1	1.67	.5490			
T+	3.67	.0001	.0002		
T3	3.67	.0001	.0002	1.0000	

Table 4. Significant Difference in the Effectiveness of Aloe vera and Honey Mixture as a Natural Tissue Fixative and the Control for Tissue Fixation in Histopathology Across Processing Quality

Source	*SS*	*df*	*MS*	*F*	*p-value*
Treatment	2.463	3	0.8211	6.16	.0179
Error	1.067	8	0.1333		
Total	3.530	11			

Table 5. Significant Difference in the Effectiveness of Aloe vera and Honey Mixture as a Natural Tissue Fixative and the Control for Tissue Fixation in Histopathology Across Tissue Block Integrity

Source	*SS*	*df*	*MS*	*F*	*p-value*
Treatment	0.010	3	0.0033	0.03	.9940
Error	1.040	8	0.1300		
Total	1.050	11			

Table 6. Significant Difference in the Effectiveness of Aloe vera and Honey Mixture as a Natural Tissue Fixative and the Control for Tissue Fixation in Histopathology Across Sectioning Qualitiy

Source	*SS*	*df*	*MS*	*F*	*p-value*
Treatment	0.667	3	0.2222	8.33	.0076
Error	0.213	8	0.0267		
Total	0.880	11			

Table 6.1. Post hoc analysis using p-values for pairwise t-test

p-values for pairwise t-tests		**T1**	**T2**	**T3**	**T+**
		2.33	**2.40**	**2.80**	**2.87**
T1	2.33				

T2	2.40	.6305			
T3	2.80	.0081	.0171		
T+	2.87	.0039	.0081	.6305	

Table 7. Significant Difference in the Effectiveness of Aloe vera and Honey Mixture as a Natural Tissue Fixative and the Control for Tissue Fixation in Histopathology Across Staining Qualitiy

Source	***SS***	***df***	***MS***	***F***	***p-value***
Treatment	0.320	3	0.1067	0.41	.7536
Error	2.107	8	0.2633		
Total	2.427	11			

Table 8. Significant Difference in the Effectiveness of Aloe vera and Honey Mixture as a Natural Tissue Fixative and the Control for Tissue Fixation in Histopathology Across Overall Morphology

Source	***SS***	***df***	***MS***	***F***	***p-value***
Treatment	0.810	3	0.2700	1.80	.2250
Error	1.200	8	0.1500		
Total	2.010	11			

Discussion

The primary finding is the marked difference in tissue morphology across the tested Aloe vera and honey fixative ratios. Treatments with higher Aloe vera proportions (T1 and T2), yielding mean overall morphology scores of 2.467 each, consistently resulted in "Fair" tissue preservation. This suggests that increasing Aloe vera concentration alone, at the tested levels, does not improve tissue integrity. This aligns with trends in cited studies, indicating Aloe vera's limited fixation capability compared to formalin or honey-rich solutions.

Conversely, the treatment with a greater honey concentration (T3 – 75% honey, 25% Aloe vera) showed a higher mean overall morphology score of 2.800, interpreted as "Good." This highlights honey's significant role in preservation within this natural mix. This supports existing research attributing honey's effectiveness to its high sugar content and low pH.

The ANOVA of overall morphology scores yielded a non-significant p-value of 0.2250 ($F(3, 8) = 1.80$). This indicates that the observed differences in mean morphology scores between treatment groups are not statistically significant at the 0.05 level. Thus, we cannot definitively conclude that the different Aloe vera to honey ratios had a statistically significant impact on overall tissue morphology in this experiment. This could be due to sample size or inherent variability.

Despite the non-significant ANOVA for overall morphology, the consistent trend of better preservation with higher honey concentration warrants attention. This descriptive

trend suggests a relationship between increased honey and improved tissue fixation quality in this natural combination. The "Good" preservation in T3, comparable to formalin in cited studies, further emphasizes honey's potential as a key component in natural fixatives.

Conclusion

The major finding is that a higher concentration of honey (75% in Treatment 3) resulted in "Good" overall tissue morphology, a level of preservation suggested by cited studies to be comparable to formalin. Conversely, treatments with a higher proportion of Aloe vera (Treatments 1 and 2) yielded only "Fair" tissue preservation, indicating that Aloe vera alone, at the tested concentrations, is not sufficient for optimal fixation. While the ANOVA analysis of overall morphology did not reveal statistically significant differences between the treatment groups, the consistent trend of improved preservation with increased honey concentration suggests a biologically relevant effect.

Future research should prioritize the investigations on expanding tissue analysis to include various types with different cellular structures to thoroughly test the versatility of Aloe vera–honey fixatives. Additionally, research should investigate the addition of other natural agents readily available in the Philippines (e.g., vinegar, citrus extracts, local plant-based preservatives) to potentially enhance their fixative properties. Future researchers should also assess the compatibility of Aloe vera–honey formulations (alone or combined) with standard histological stains like Hematoxylin and Eosin (H&E). Finally, a thorough cost-analysis comparing these natural fixatives against traditional formalin, specifically considering local sourcing of Aloe vera through cultivation and sustainable beekeeping practices in the Ilocos Region of the Philippines, is essential to evaluate their economic feasibility and potential for local adoption.

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vera (Aloe barbadensis Miller). *Journal of Agriculture and Food Research*, *21*, 101785. https://doi.org/10.1016/j.jafr.2025.101785

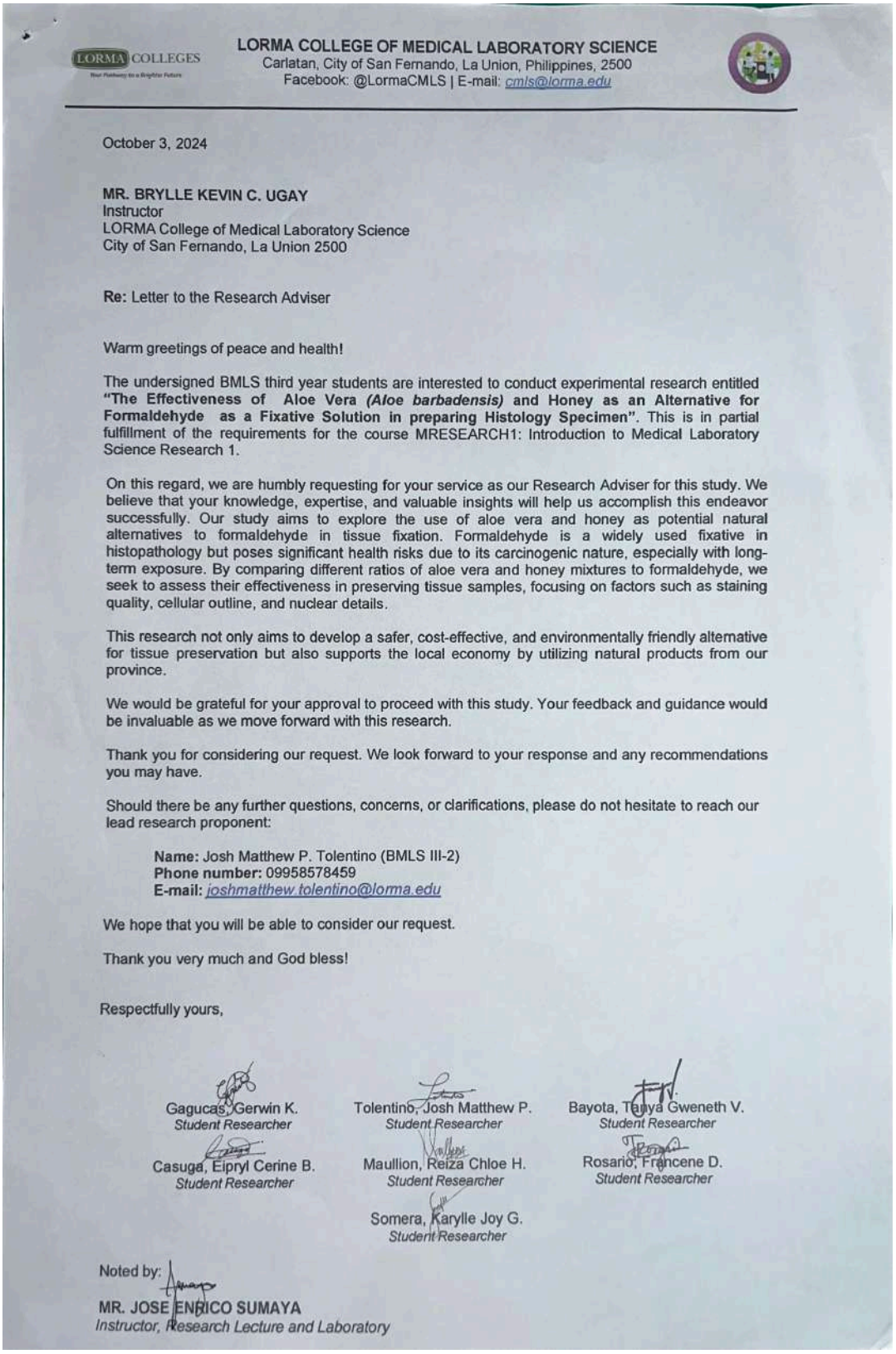
Zanini C., Gerbaudo, E., Ercole, E., Vendramin, A., & Forni, M. (2012b). Evaluation of two commercial and three home-made fixatives for the substitution of formalin: a formaldehyde–free laboratory is possible. Environmental Health, 11(1). https://doi.org/10.1186/1476-069x-11-59

Zhang, L., Freeman, L. E., Nakamura, J., Hecht, S. S., Vandenberg, J. J., Smith, M. T., & Sonawane, B. R. (2010). Formaldehyde and leukemia: epidemiology, potential mechanisms, and implications for risk assessment. *Environmental and Molecular Mutagenesis, 51*(3), 181–191. https://doi.org/10.1002/em.20534

Appendices

Appendix A

Letter of Intent to the Faculty Research Adviser



LORMA COLLEGES

LORMA COLLEGE OF MEDICAL LABORATORY SCIENCE
Carlatan, City of San Fernando, La Union, Philippines, 2500
Facebook: @LormaCMLS | E-mail: cmls@lorma.edu

October 3, 2024

MR. BRYLLE KEVIN C. UGAY
Instructor
LORMA College of Medical Laboratory Science
City of San Fernando, La Union 2500

Re: Letter to the Research Adviser

Warm greetings of peace and health!

The undersigned BMLS third year students are interested to conduct experimental research entitled **"The Effectiveness of Aloe Vera *(Aloe barbadensis)* and Honey as an Alternative for Formaldehyde as a Fixative Solution in preparing Histology Specimen"**. This is in partial fulfillment of the requirements for the course MRESEARCH1: Introduction to Medical Laboratory Science Research 1.

On this regard, we are humbly requesting for your service as our Research Adviser for this study. We believe that your knowledge, expertise, and valuable insights will help us accomplish this endeavor successfully. Our study aims to explore the use of aloe vera and honey as potential natural alternatives to formaldehyde in tissue fixation. Formaldehyde is a widely used fixative in histopathology but poses significant health risks due to its carcinogenic nature, especially with long-term exposure. By comparing different ratios of aloe vera and honey mixtures to formaldehyde, we seek to assess their effectiveness in preserving tissue samples, focusing on factors such as staining quality, cellular outline, and nuclear details.

This research not only aims to develop a safer, cost-effective, and environmentally friendly alternative for tissue preservation but also supports the local economy by utilizing natural products from our province.

We would be grateful for your approval to proceed with this study. Your feedback and guidance would be invaluable as we move forward with this research.

Thank you for considering our request. We look forward to your response and any recommendations you may have.

Should there be any further questions, concerns, or clarifications, please do not hesitate to reach our lead research proponent:

Name: Josh Matthew P. Tolentino (BMLS III-2)
Phone number: 09958578459
E-mail: joshmatthew.tolentino@lorma.edu

We hope that you will be able to consider our request.

Thank you very much and God bless!

Respectfully yours,

Gagucas, Gerwin K.
Student Researcher

Tolentino, Josh Matthew P.
Student Researcher

Bayota, Tanya Gweneth V.
Student Researcher

Casuga, Eipryl Cerine B.
Student Researcher

Maullion, Reiza Chloe H.
Student Researcher

Rosario, Francene D.
Student Researcher

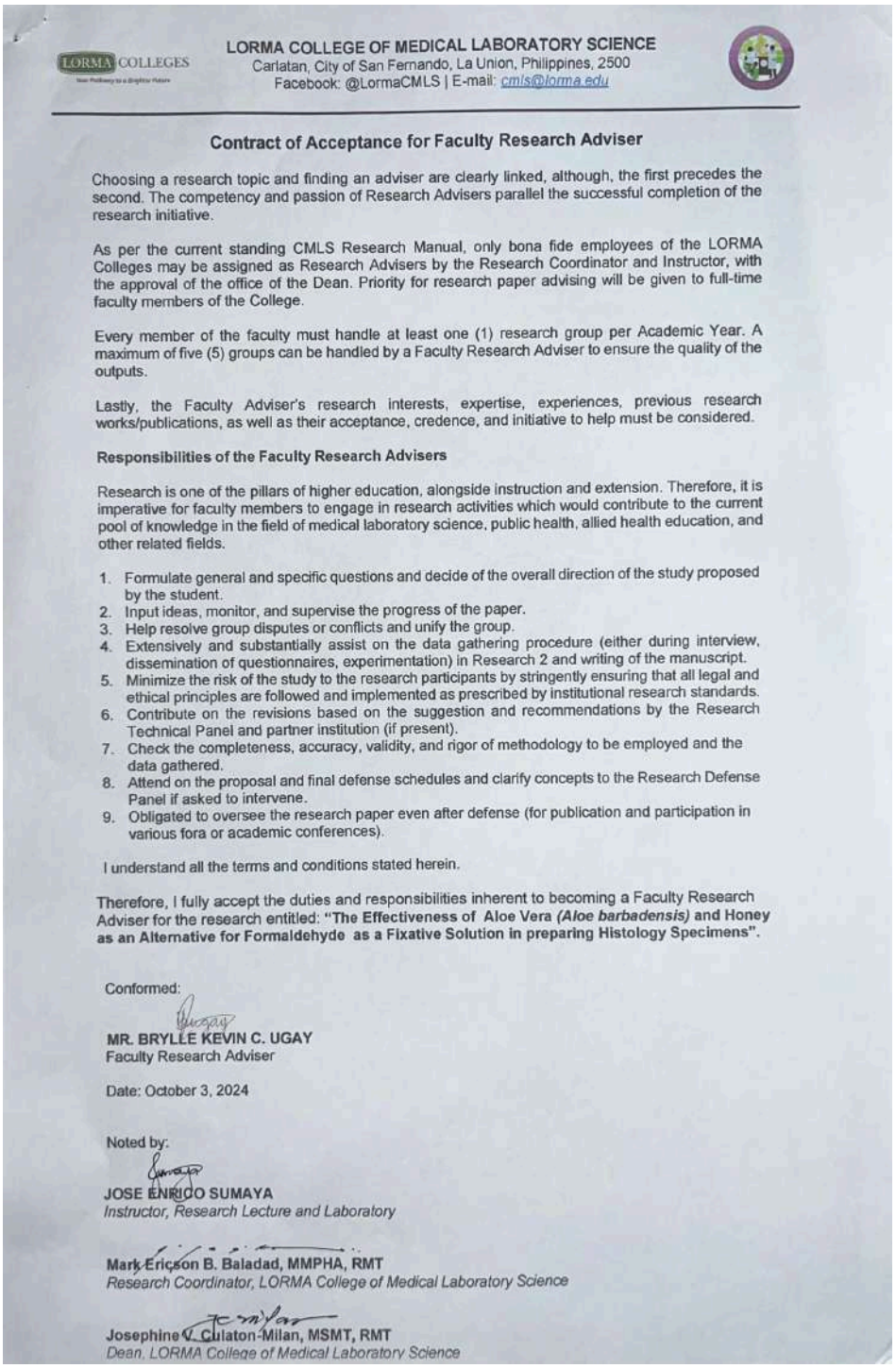
Somera, Karylle Joy G.
Student Researcher

Noted by:

MR. JOSE ENRICO SUMAYA
Instructor, Research Lecture and Laboratory

Appendix B

Contract of Acceptance for Faculty Research Adviser



LORMA COLLEGES

LORMA COLLEGE OF MEDICAL LABORATORY SCIENCE
Carlatan, City of San Fernando, La Union, Philippines, 2500
Facebook: @LormaCMLS | E-mail: cmls@lorma.edu

Contract of Acceptance for Faculty Research Adviser

Choosing a research topic and finding an adviser are clearly linked, although, the first precedes the second. The competency and passion of Research Advisers parallel the successful completion of the research initiative.

As per the current standing CMLS Research Manual, only bona fide employees of the LORMA Colleges may be assigned as Research Advisers by the Research Coordinator and Instructor, with the approval of the office of the Dean. Priority for research paper advising will be given to full-time faculty members of the College.

Every member of the faculty must handle at least one (1) research group per Academic Year. A maximum of five (5) groups can be handled by a Faculty Research Adviser to ensure the quality of the outputs.

Lastly, the Faculty Adviser's research interests, expertise, experiences, previous research works/publications, as well as their acceptance, credence, and initiative to help must be considered.

Responsibilities of the Faculty Research Advisers

Research is one of the pillars of higher education, alongside instruction and extension. Therefore, it is imperative for faculty members to engage in research activities which would contribute to the current pool of knowledge in the field of medical laboratory science, public health, allied health education, and other related fields.

1. Formulate general and specific questions and decide of the overall direction of the study proposed by the student.
2. Input ideas, monitor, and supervise the progress of the paper.
3. Help resolve group disputes or conflicts and unify the group.
4. Extensively and substantially assist on the data gathering procedure (either during interview, dissemination of questionnaires, experimentation) in Research 2 and writing of the manuscript.
5. Minimize the risk of the study to the research participants by stringently ensuring that all legal and ethical principles are followed and implemented as prescribed by institutional research standards.
6. Contribute on the revisions based on the suggestion and recommendations by the Research Technical Panel and partner institution (if present).
7. Check the completeness, accuracy, validity, and rigor of methodology to be employed and the data gathered.
8. Attend on the proposal and final defense schedules and clarify concepts to the Research Defense Panel if asked to intervene.
9. Obligated to oversee the research paper even after defense (for publication and participation in various fora or academic conferences).

I understand all the terms and conditions stated herein.

Therefore, I fully accept the duties and responsibilities inherent to becoming a Faculty Research Adviser for the research entitled: **"The Effectiveness of Aloe Vera *(Aloe barbadensis)* and Honey as an Alternative for Formaldehyde as a Fixative Solution in preparing Histology Specimens".**

Conformed:

MR. BRYLLE KEVIN C. UGAY
Faculty Research Adviser

Date: October 3, 2024

Noted by:

JOSE ENRICO SUMAYA
Instructor, Research Lecture and Laboratory

Mark Ericson B. Baladad, MMPHA, RMT
Research Coordinator, LORMA College of Medical Laboratory Science

Josephine V. Culaton-Milan, MSMT, RMT
Dean, LORMA College of Medical Laboratory Science

Appendix C

Questionnaire For Grading Criteria

Questionnaire For Grading Criteria

Name:_____

Date:_____

Parameter	Scoring Guidelines				Score
Specimen Preparation					
	4 **Excellent**	**3** **Good**	**2** **Fair**	**1** **Poor**	
Fixation Quality	Tissue is fully fixed, showing no autolysis, shrinkage, or hardening. Nuclear membranes and chromatin are crisp, and cytoplasmic details are intact.	Tissue is mostly fixed but may have minor areas of uneven penetration or slight autolysis. Shrinkage is minimal.	Fixation is inconsistent, with areas of autolysis, moderate shrinkage, or improper penetration. Chromatin is less distinct.	Fixation is poor, with widespread autolysis, shrinkage, or incomplete penetration. Cellular details are lost	
Processing Quality	The tissue has been processed very well. It shows proper dehydration and clearing, without any signs of remaining moisture or clearing agent.	The tissue processing is generally good. There might be some small areas where dehydration or clearing isn't consistent, but it's not enough to cause significant problems.	The tissue processing shows noticeable inconsistencies. This has led to some soft spots within the tissue block.	The tissue processing was inadequate. This has resulted in uneven clearing, retained moisture, or insufficient paraffin infiltration.	
Tissue Block Integrity	Block is firm, smooth, and free of cracks or crumbling, ensuring excellent stability and usability.	Block has minor surface cracks or slightly uneven texture but remains stable for sectioning	Block shows moderate defects such as visible cracks, brittleness, or crumbling, affecting handling.	Block is severely compromised, with extensive cracking, crumbling, or instability that renders it	

				unusable	
Sectioning Quality	Sections are even, thin, and free from tears, folds, or compression artifacts. No irregularities are visible under the microscope.	Sections are mostly smooth, with slight tears or compression artifacts that do not obscure critical tissue details.	Sections show moderate defects, such as tearing, folding, or compression, that partially obscure tissue details.	Sections are unusable, with extensive folds, tears, or compression artifacts obscuring tissue details entirely.	
Staining Quality	Staining is uniform, with excellent differentiation between nuclei and cytoplasm. No blotches or faded areas are observed.	Staining is mostly uniform, with minor imperfections like faint areas or slight uneven application of dyes.	Staining is uneven, with visible blotches, weak nuclear-cytoplasmic contrast, or incomplete coverage in some areas.	Staining is inadequate, with unstained areas, excessive blotching, or over-saturation that obscures cellular structures. .	
Overall Morphology	Tissue is fully intact, showing no breaks, gaps, or distortions. All structural components are preserved and clearly visible.	Tissue is mostly intact, with minor tears, gaps, or slightly misaligned layers that do not hinder analysis.	Tissue shows moderate damage, such as separation of layers or partial loss of structural integrity, affecting visualization.	Tissue is severely damaged, distorted, or fragmented, making reliable analysis impossible.	
Total					

Appendix D
Request Letter for Pig Liver Specimen

LORMA COLLEGES

LORMA COLLEGE OF MEDICAL LABORATORY SCIENCE
Carlatan, City of San Fernando, La Union, Philippines, 2500
Facebook: @LormaCMLS | E-mail: cmls@lorma.edu

March 11, 2025

Office of the Slaughterhouse
San Fernando City, La Union 2500

Warm greetings of peace and health!

The undersigned BMLS third year students will conduct experimental research entitled **"Evaluating the Synergistic Effects of Aloe vera (*Aloe Barbadensis)* and Honey as Natural Fixatives for Tissue Fixation in Histopathology".** This is in fulfillment of the requirements for the course MRESEARCH2: Medical Laboratory Science Research Paper Writing and Presentation.

Our research aims to explore the potential of natural fixatives in histopathology, specifically the combination of Aloe vera and honey. This study seeks to evaluate their effectiveness in tissue fixation, comparing their properties to traditional chemical fixatives. We believe this research could contribute significantly to the field of histopathology by introducing sustainable and non-toxic alternatives for tissue preservation.

As part of our research, we require **600 grams of pig liver** as a specimen for tissue fixation testing. The specimen will be sourced from the San Fernando City Slaughterhouse. In this regard, we humbly request your permission to obtain or purchase the required pig liver from your facility.

Your support in providing the specimen will be greatly appreciated. We are open to any procedures or requirements necessary and look forward to your response.

Should there be any further questions, concerns, or clarifications, please do not hesitate to reach our lead research proponent:

Name: Josh Matthew P. Tolentino (BMLS III-2)
Phone number: 09958578459
E-mail: joshmatthew.tolentino@lorma.edu

We hope that you will be able to consider our request. Thank you very much and God bless!

Respectfully yours,

Gagucas, Gerwin K.
Student Researcher

Tolentino, Josh Matthew P.
Student Researcher

Bayota, Tanya Gweneth V.
Student Researcher

Casuga, Eipryl Cerine B.
Student Researcher

Maullion, Reiza Chloe H.
Student Researcher

Rosario, Francene D.
Student Researcher

Somera, Karylle Joy G.
Student Researcher

Appendix E

Sanitary Permit to Operate – Slaughterhouse of San Fernando City

Republic of the Philippines
CITY OF SAN FERNANDO
OFFICE OF THE CITY HEALTH OFFICER

SANITARY PERMIT TO OPERATE

Issued to

SLAUGTHERHOUSE OF CITY OF SAN FERNANDO, LA UNION
(Registered Name)

SLAUGTHERHOUSE
(Type of Establishment / Nature of Business)

CITY GOVERNMENT OF CITY OF SAN FERNANDO, LA UNION
(Owner / Operator

Address **BRGY. TANQUI, CFS, LU**

Sanitary Permit No: 001 DATE ISSUED: FEBRUARY 12, 2025

Date of Expiration: DECEMBER 31, 2025

This permit is not transferable and will be revoked for violation of the Sanitary Rules, Laws or Regulation of P.D. 522 & P.D. 856 and Pertinent Local Ordinances.

Recommending Approval: **ENGR. EDUARDO D. FLORA, EE**
Sanitation Inspector I

Approved : **MICHAEL C. BANGLOY, MD**
City Health Officer II

Noted : **HON. HERMENEGILDO A. GUALBERTO**
City Mayor

THIS MUST BE DISPLAYED CONSPICUOUSLY AT THE PLACE OF BUSINESS

Appendix F

Certificate of Animal Liver Specimen Identification

Province of La Union
City Government of San Fernando
OFFICE OF THE CITY VETERINARIAN

BAGONG PILIPINAS

CERTIFICATE

This office certifies that the specie of animal liver collected from the City Slaughterhouse for the research of the Lorma College of Medical Laboratory Science students is Sus scrofa domesticus.

For any legal intent.

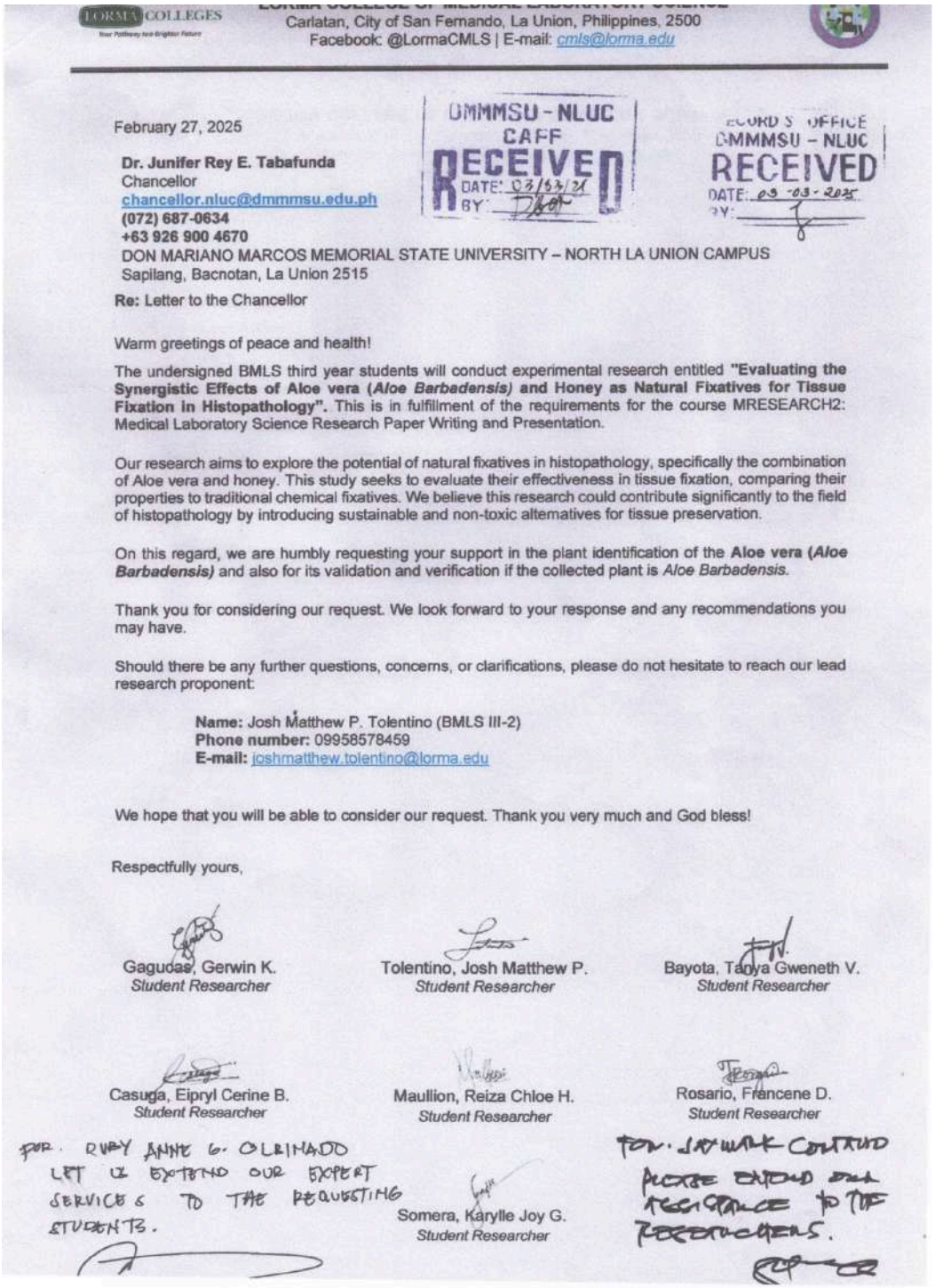
FLOSIE P. DECENA
City Veterinarian

SAN FERNANDO TAYO

P. Burgos St., Brgy. Tanqui
City of San Fernando, Province of La Union 2500
(072)607-8949/CP No. 09189273384
vet@sanfernandocity.gov.ph
www.sanfernandocity.gov.ph

Appendix G

Request Letter for Plant Identification



LORMA COLLEGES
Carlatan, City of San Fernando, La Union, Philippines, 2500
Facebook: @LormaCMLS | E-mail: cmls@lorma.edu

DMMMSU - NLUC
CAFF
RECEIVED
DATE: 03/03/25
BY:

RECORDS OFFICE
DMMMSU - NLUC
RECEIVED
DATE: 03-03-2025
BY:

February 27, 2025

Dr. Junifer Rey E. Tabafunda
Chancellor
chancellor.nluc@dmmmsu.edu.ph
(072) 687-0634
+63 926 900 4670
DON MARIANO MARCOS MEMORIAL STATE UNIVERSITY – NORTH LA UNION CAMPUS
Sapilang, Bacnotan, La Union 2515

Re: Letter to the Chancellor

Warm greetings of peace and health!

The undersigned BMLS third year students will conduct experimental research entitled **"Evaluating the Synergistic Effects of Aloe vera (*Aloe Barbadensis)* and Honey as Natural Fixatives for Tissue Fixation in Histopathology".** This is in fulfillment of the requirements for the course MRESEARCH2: Medical Laboratory Science Research Paper Writing and Presentation.

Our research aims to explore the potential of natural fixatives in histopathology, specifically the combination of Aloe vera and honey. This study seeks to evaluate their effectiveness in tissue fixation, comparing their properties to traditional chemical fixatives. We believe this research could contribute significantly to the field of histopathology by introducing sustainable and non-toxic alternatives for tissue preservation.

On this regard, we are humbly requesting your support in the plant identification of the **Aloe vera (*Aloe Barbadensis)*** and also for its validation and verification if the collected plant is *Aloe Barbadensis*.

Thank you for considering our request. We look forward to your response and any recommendations you may have.

Should there be any further questions, concerns, or clarifications, please do not hesitate to reach our lead research proponent:

Name: Josh Matthew P. Tolentino (BMLS III-2)
Phone number: 09958578459
E-mail: joshmatthew.tolentino@lorma.edu

We hope that you will be able to consider our request. Thank you very much and God bless!

Respectfully yours,

Gagudas, Gerwin K.
Student Researcher

Tolentino, Josh Matthew P.
Student Researcher

Bayota, Tanya Gweneth V.
Student Researcher

Casuga, Eipryl Cerine B.
Student Researcher

Maullion, Reiza Chloe H.
Student Researcher

Rosario, Francene D.
Student Researcher

FOR. RUBY ANNE G. OLIMADO
LET US EXTEND OUR EXPERT SERVICES TO THE REQUESTING STUDENTS.

Somera, Karylle Joy G.
Student Researcher

FOR. JAYMARK CONTRERAS
PLEASE EXTEND OUR ASSISTANCE TO THE RESEARCHERS.

Appendix H
Identification Certificate of Plant Material

DON MARIANO MARCOS MEMORIAL STATE UNIVERSITY
NORTH LA UNION CAMPUS, Bacnotan, La Union, Philippines
COLLEGE OF AGROFORESTRY AND FORESTRY
www.dmmmsu.edu.ph | +63-938-032-6976 | caff.nluc@dmmmsu.edu.ph

RECORD'S OFFICE
DMMMSU – NLUC
RELEASED
DATE: 03-05-2025
BY:

IDENTIFICATION CERTIFICATE OF PLANT MATERIAL

This is to certify that **GERWIN K. GAGUCAS, JOSH MATTHEW P. TOLENTINO, TANYA GWENETH V. BAYOTA, EIPRYL CERINE B. CASUGA, REIZA CHLOE H. MAULLION, FRANCENE D. ROSARIO,** and **KARYLLE JOY G. SOMERA,** of the College of Medical Laboratory Science, Lorma Colleges, City of San Fernando, La Union, have brought plant species for proper authentic identification. After a thorough and closer examination on the morphological and botanical characteristics of the specimen, it was identified and described as follows.

Common Name - Aloe vera
Scientific Name - *Aloe barbadensis* (Miller)
Family Name - Asphodelaceae

This certification is issued to **Gerwin K. Gagucas, Josh Matthew P. Tolentino, Tanya Gweneth V. Bayota, Eipryl Cerine B. Casuga, Reiza Chloe H. Maullion, Francene D. Rosario,** and **Karylle Joy G. Somera** for all legal intentions and purposes.

Issued this 5th day of March 2025, College of Agroforestry and Forestry, Don Mariano Mariano Marcos Memorial State University, North La Union Campus, Bacnotan, La Union.

Prepared and examined by:

FOR. RUBY ANNE G. OLBINADO
Dendrologist/Faculty, CAFF

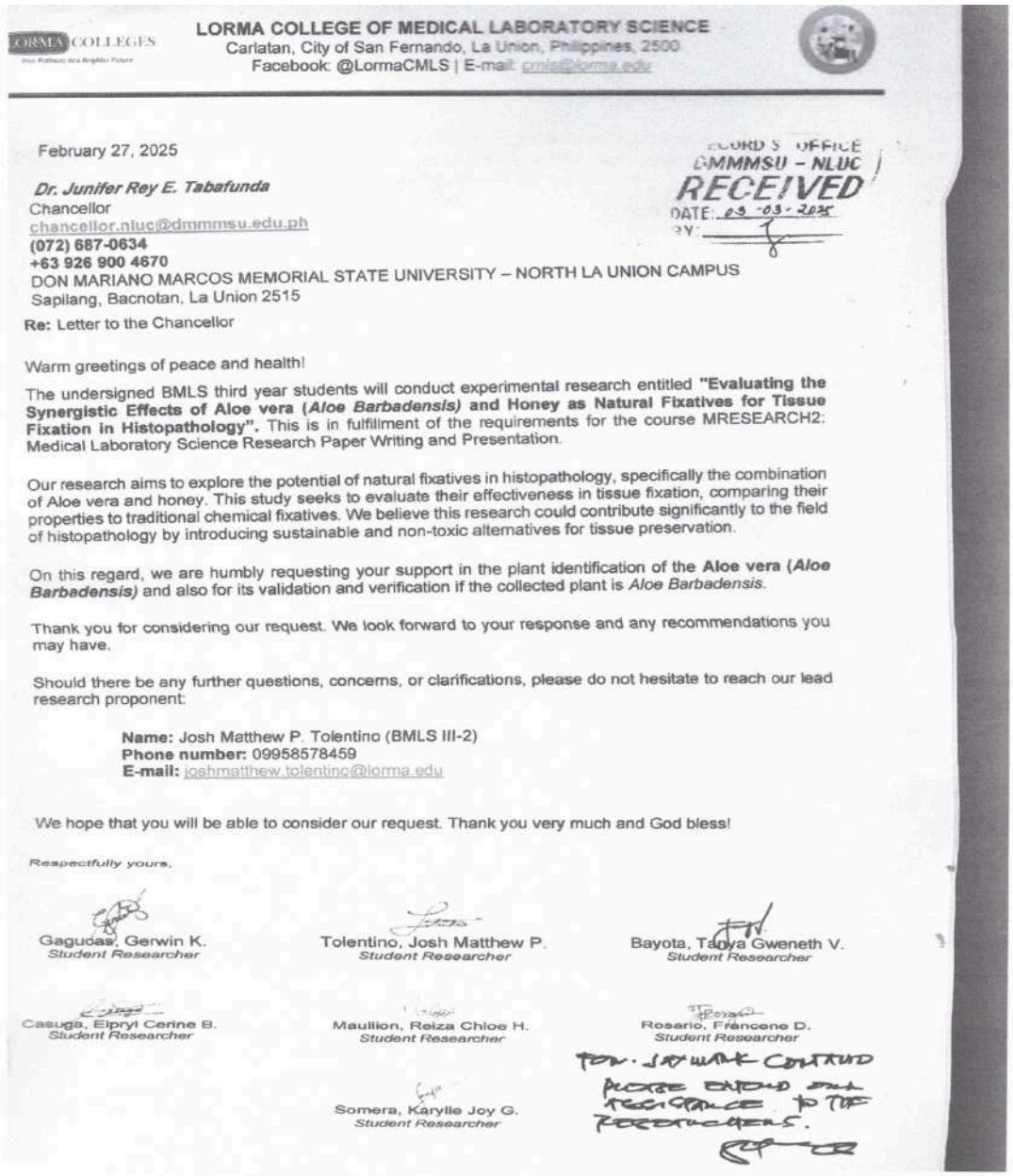
Noted:

FOR. JAY MARK G. CORTADO
Dean, CAFF

DR. JUNIFER REY E. TABAFUNDA
Chancellor

Appendix I

Letter of Request for Plant Identification and Verification



LORMA COLLEGES

LORMA COLLEGE OF MEDICAL LABORATORY SCIENCE
Carlatan, City of San Fernando, La Union, Philippines, 2500
Facebook: @LormaCMLS | E-mail: cmls@lorma.edu

RECORDS OFFICE
DMMMSU - NLUC
RECEIVED
DATE: 03-03-2025
BY:

February 27, 2025

Dr. Junifer Rey E. Tabafunda
Chancellor
chancellor.nluc@dmmmsu.edu.ph
(072) 687-0634
+63 926 900 4670
DON MARIANO MARCOS MEMORIAL STATE UNIVERSITY – NORTH LA UNION CAMPUS
Sapilang, Bacnotan, La Union 2515

Re: Letter to the Chancellor

Warm greetings of peace and health!

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Thank you for considering our request. We look forward to your response and any recommendations you may have.

Should there be any further questions, concerns, or clarifications, please do not hesitate to reach our lead research proponent:

Name: Josh Matthew P. Tolentino (BMLS III-2)
Phone number: 09958578459
E-mail: joshmatthew.tolentino@lorma.edu

We hope that you will be able to consider our request. Thank you very much and God bless!

Respectfully yours,

Gagudas, Gerwin K. — *Student Researcher*
Tolentino, Josh Matthew P. — *Student Researcher*
Bayota, Tanya Gweneth V. — *Student Researcher*
Casuga, Eipryl Cerine B. — *Student Researcher*
Maullion, Reiza Chloe H. — *Student Researcher*
Rosario, Francene D. — *Student Researcher*
Somera, Karylle Joy G. — *Student Researcher*

FOR. SAY WORK CONTRUD
PLEASE EXTEND OUR
REGARDS TO THE
RESPONDENTS.

LORMA COLLEGES

LORMA COLLEGE OF MEDICAL LABORATORY SCIENCE
Carlatan, City of San Fernando, La Union, Philippines, 2500
Facebook: @LormaCMLS | E-mail: cmls@lorma.edu

Noted by:

Brylle Kevin C. Ugay, RMT
Faculty Research Adviser

Jose Enrico M. Sumaya, RMT
Instructor, Research Lecture and Laboratory

Mark Erioson B. Baladad, MMPHA, RMT
Research Coordinator, LORMA College of Medical Laboratory Science

Josephine V. Culaton-Milan, MSMT, RMT
Dean, LORMA College of Medical Laboratory Science

Appendix J
Identification Certificate of Plant Material

DON MARIANO MARCOS MEMORIAL STATE UNIVERSITY
NORTH LA UNION CAMPUS, Bacnotan, La Union, Philippines
COLLEGE OF AGROFORESTRY AND FORESTRY
www.dmmmsu.edu.ph | +63-938-032-6976 | caff.nluc@dmmmsu.edu.ph

RELEASED
DMMMSU - NLUC
DATE: 03 DEC 2024
BY:

IDENTIFICATION CERTIFICATE OF PLANT MATERIAL

This is to certify that **GERWIN K. GAGUCAS, JOSH MATTHEW P. TOLENTINO, TANYA GWENETH V. BAYOTA, EIPRYL CERINE B. CASUGA, REIZA CHLOE H. MAULLION, FRANCENE D. ROSARIO,** and **KARYLLE JOY G. SOMERA,** of the College of Medical Laboratory Science, Lorma Colleges, City of San Fernando, La Union, have brought plant species for proper authentic identification. After a thorough and closer examination on the morphological and botanical characteristics of the specimen, it was identified and described as follows.

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Family Name - Asphodelaceae

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Issued this 5th day of March 2025, College of Agroforestry and Forestry, Don Mariano Mariano Marcos Memorial State University, North La Union Campus, Bacnotan, La Union.

Prepared and examined by:

FOR. RUBY ANNE C. OLBINADO
Dendrologist/Faculty, CAFF

DMMMSU - NLUC
CAFF
RELEASED
DATE: 05/05/25
BY:

Noted:

FOR. JAY MARK G. CORTADO
Dean, CAFF

DR. JUNIFER REY E. TABAFUNDA
Chancellor

Appendix K

Honey Certification

HONEY CERTIFICATION

DON MARIANO MARCOS MEMORIAL STATE UNIVERSITY- NATIONAL APICULTURE RESEARCH AND DEVELOPMENT INSTITUTE

This is to certify that the honey produced and harvested from wild sunflowers (*Tithonia diversifolia*) in the province of Benguet has met the following quality standards:

- **Origin:** Buguias, Benguet, Philippines
- **Bee Specie:** European Honeybee (Apis mellifera)
- **Floral Source:** Wild Sunflowers (*Tithonia diversifolia*)
- **Harvesting Method:** Natural and Sustainable Extraction
- **Moisture Content:** 19.6%
- **Purity:** 100% Raw and Unadulterated Honey
- **Processing:** Free from Artificial Additives and Preservatives

This certification attests that the honey complies with the industry standards for premium quality and is fit for consumption.

Certified by:

DAVID T. DE CASTRO
Division Chief, Innovation and Technology Commercialization, Research & Extension and IEC Materials Development

This certification remains valid unless otherwise stated or revoked due to non-compliance with quality standards.

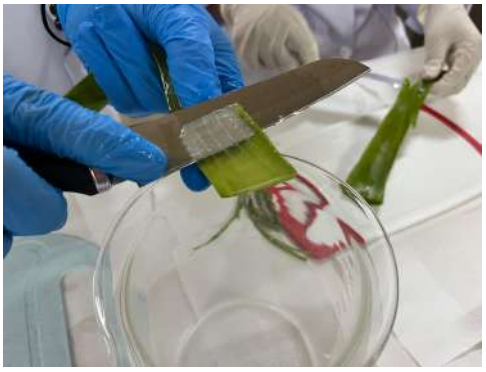
Appendix L

Pictures of Experimental Set-Ups

Aloe vera (*Aloe barbadensis*) plant collection

Washing and cleaning of aloe veras

Aloe vera Gel Extraction



Liquid Extraction From The Gel (first filtration)

Liquid Extraction From The Gel (Second Filtration)


Purchasing of Honey in DMMMSU-NLUC





Aloe Vera Plant Identification


Equipment Sterilization Using Autoclave, Antibacterial Soap, and Hypochlorite





Preparation of Aloe vera-Honey Solution








Preparation of Aloe vera-Honey Solution





Pig Liver Material Collection at City Slaughterhouse and Transporting using the Treatment Ratios of Aloe Vera-Honey Solution and Formalin

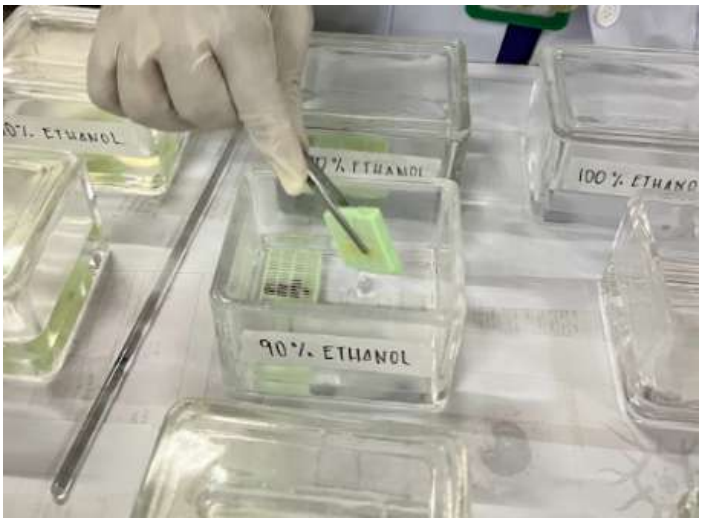




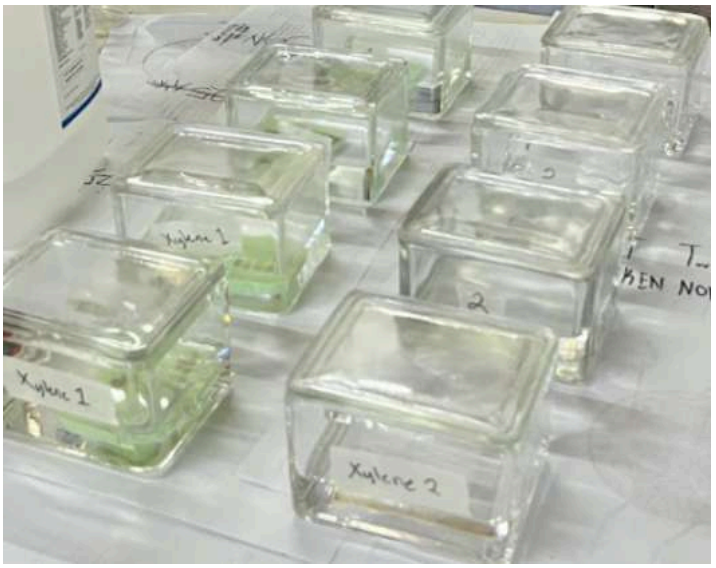

Grossing and Cutting of Pig Liver Specimens after T

Fixation of Grossed and Cut Liver Tissues into Different Treatments

Dehydration of the Different Specimens

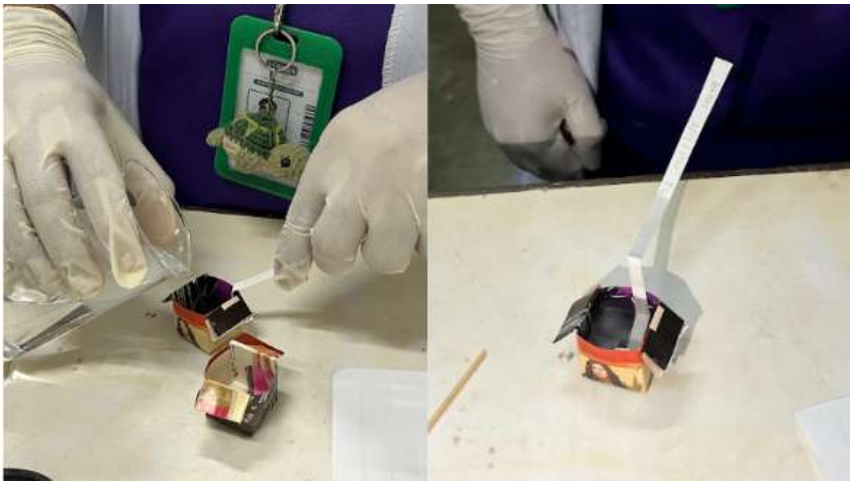


Clearing of the Different Specimens



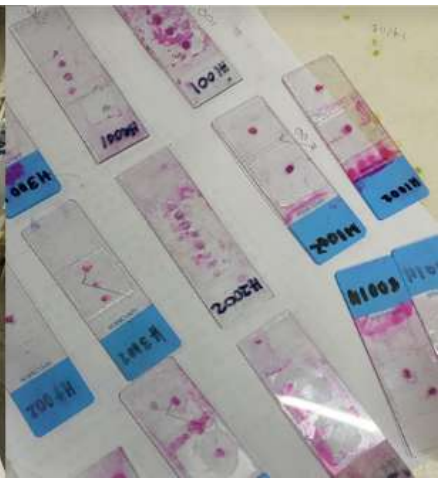
Infiltration or Impregnation Using Paraffin Wax

Embedding of the Processed Tissues Using Paper Boats

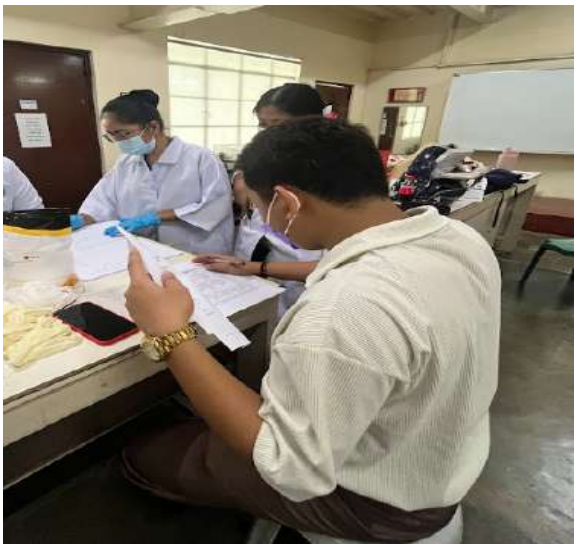


Section-Cutting of the Tissue Blocks into Ribbons


Staining and Drying of the Slides

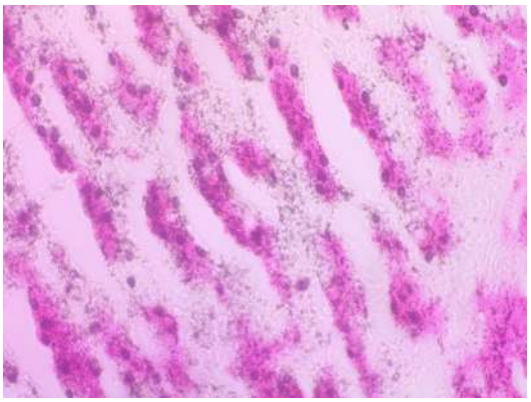



Evaluation of the Evaluators

Appendix M

H+001 (FORMALIN)



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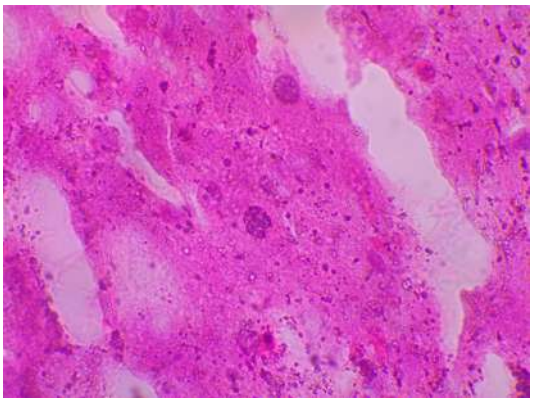

OIO.

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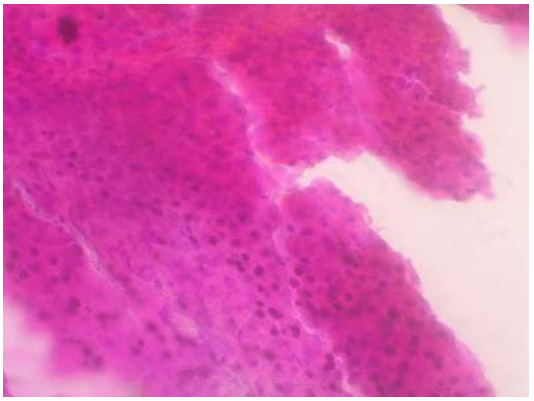

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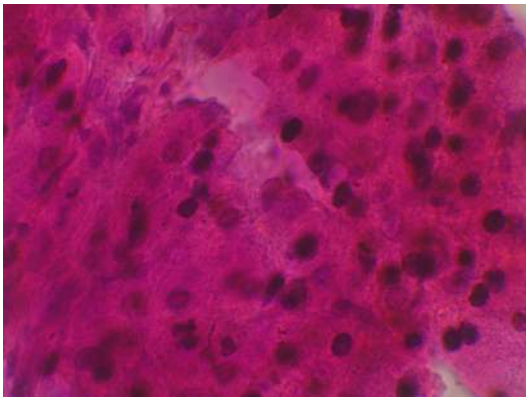

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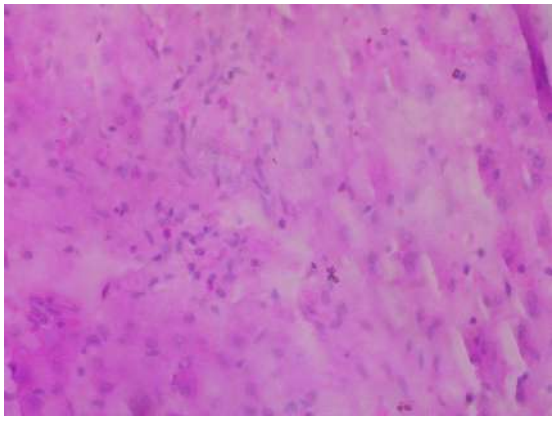

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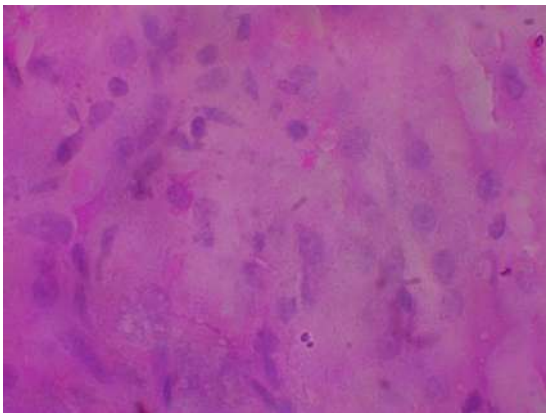

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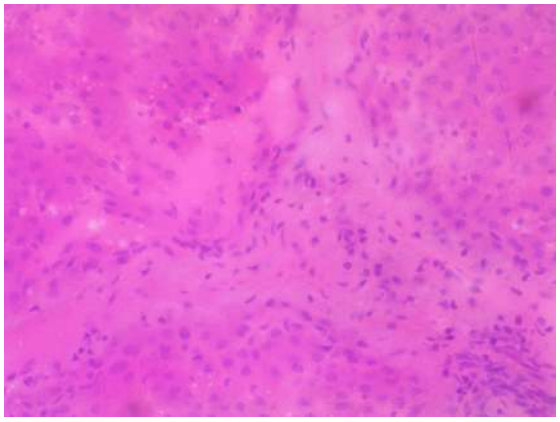
H1001 (50% Aloe Vera : 50% Honey)



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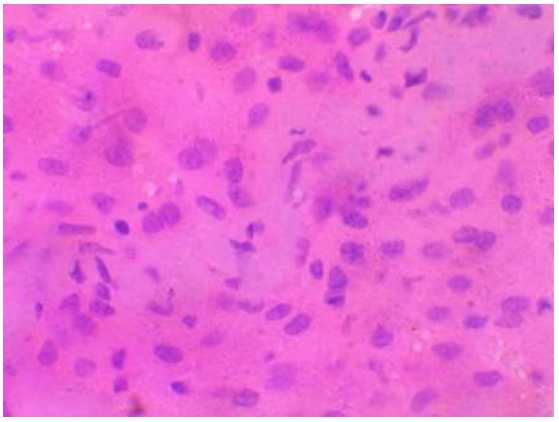

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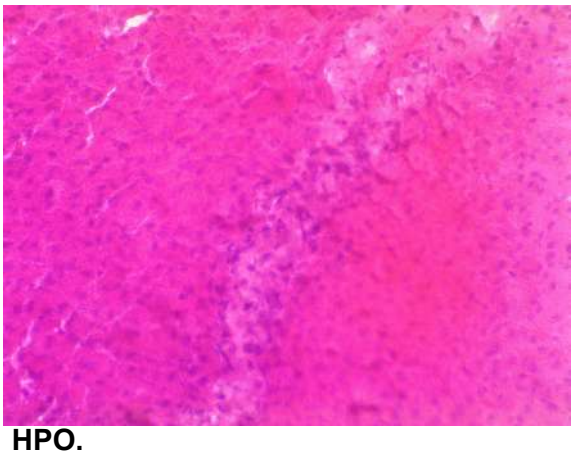
H1002



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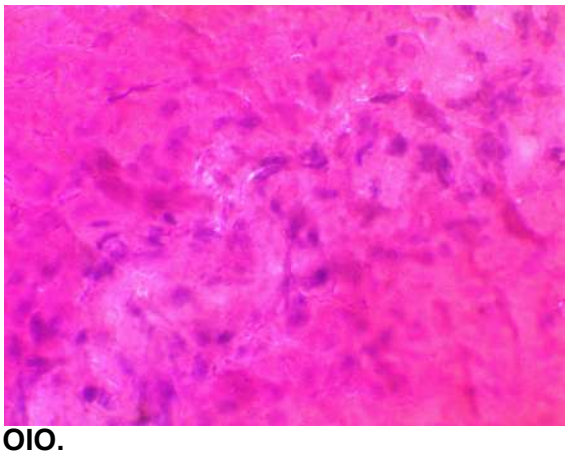

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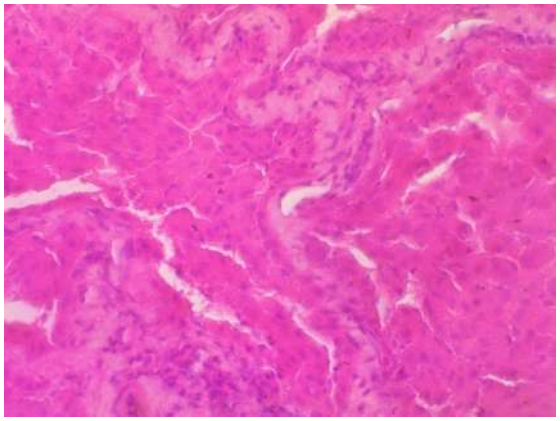

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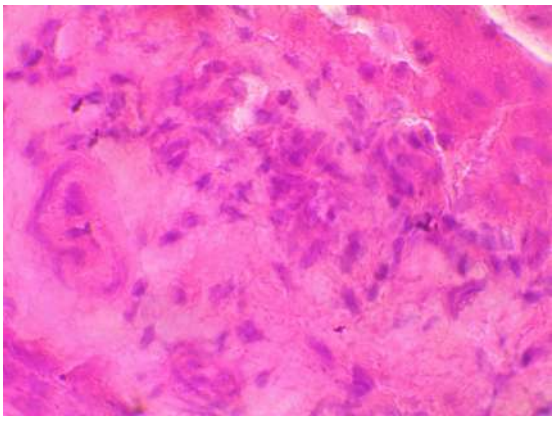

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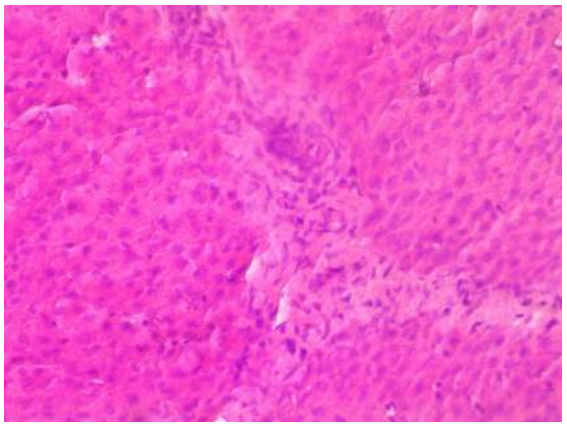
H2001 (75% Aloe Vera : 25% Honey)



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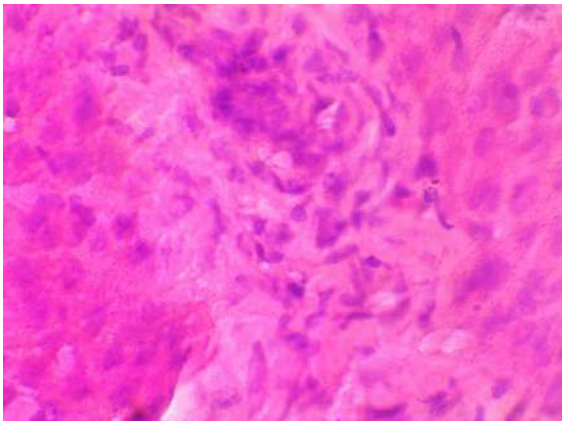

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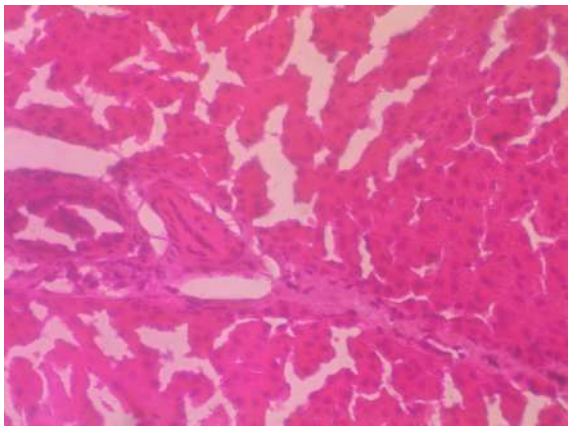

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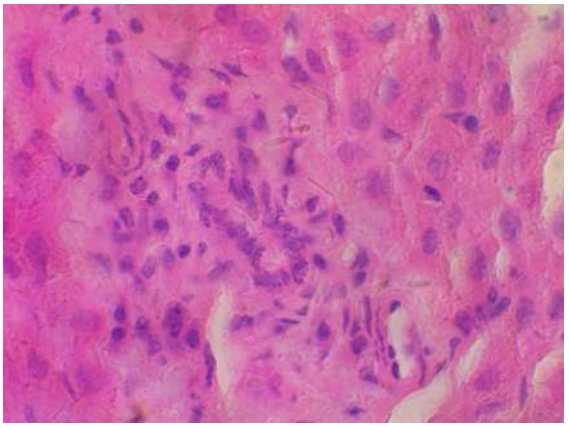

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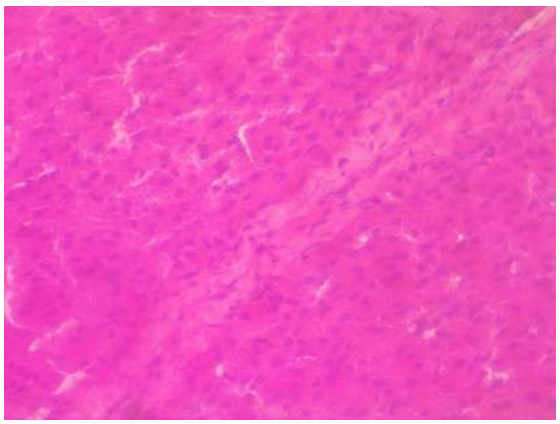

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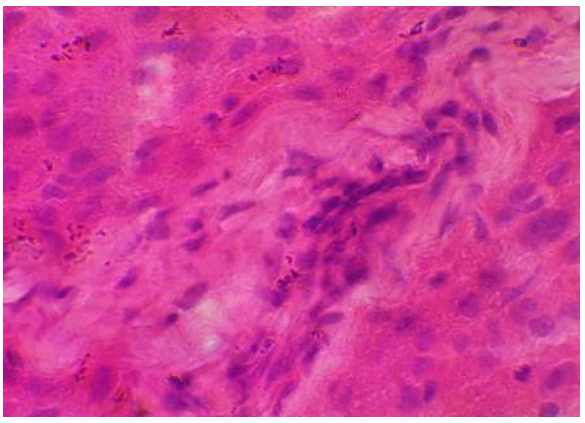
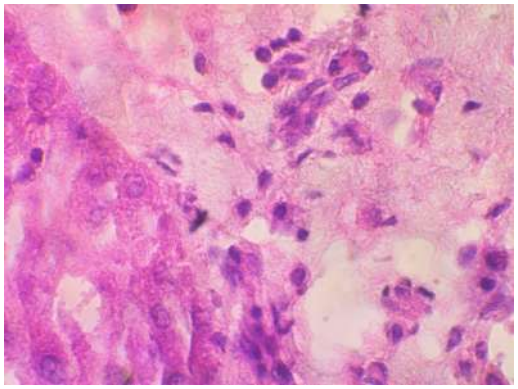


OIO

H3001 (25% Aloe Vera : 75% Honey)



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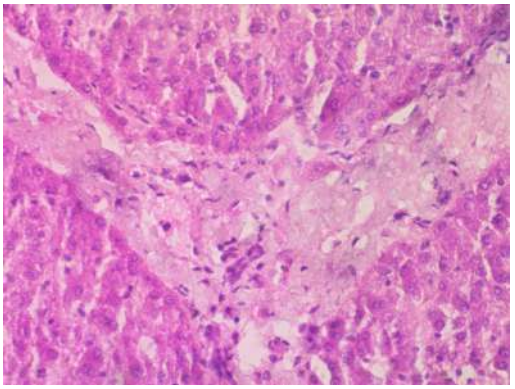

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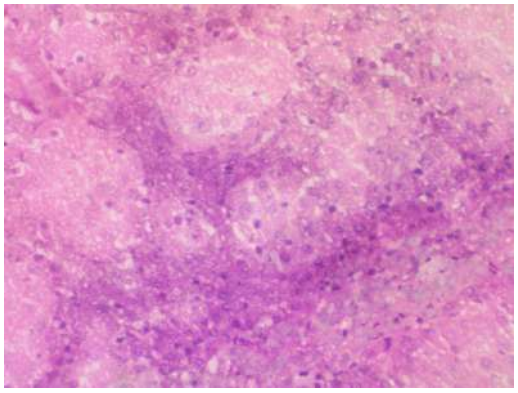
H3002



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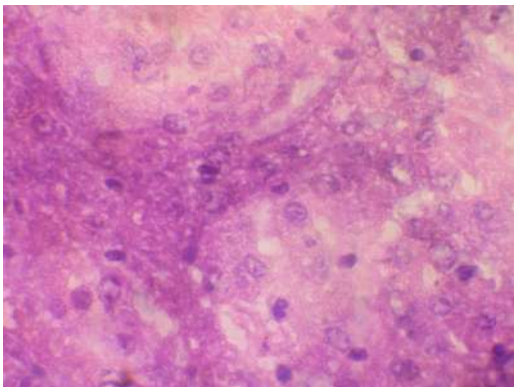

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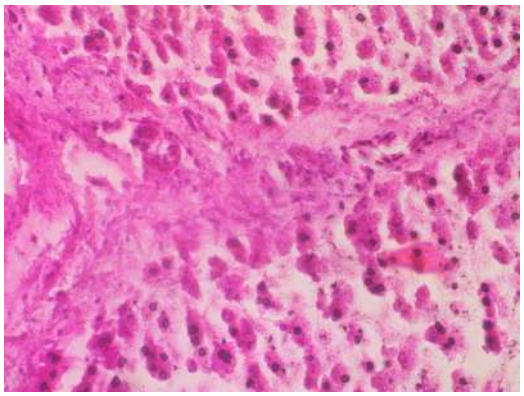
H3003



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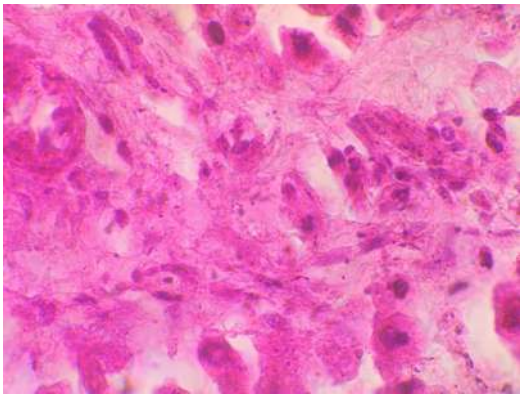


OIO.

Appendix N

Research Timetable

Title of the study: "Evaluating the Synergistic Effects of Aloe Vera (*Aloe Barbadensis*) and Honey as Natural Fixatives for Tissue Fixation in Histopathology"

Proponents: Bayota, Tanya Gweneth V., Casuga, Eipryl Cerine B., Gagucas, Gerwin K., Maullion, Reiza Chloe H., Rosario, Francene D., Somera, Karylle Joy g., Tolentino, Josh Matthew P.

Timetable	Research Task	Methodology/Strategy/Technique	Status
9/1/24	Formulation of Research Title	Face to Face Meeting	Completed
9/7/24	Proposal for Research Title	Face to Face Meeting Presentation of the studies at FB 301	Completed
10/4/24	Signing of the Research Adviser and approval by the Dean	Face to Face Meeting Consultation with the Dean	Completed
10/22/24	Pretesting of the Aloe Vera and Honey	Face to Face Meeting Laboratory work in S2 101	Completed
11/15/24	Formulation of Chapter 1	Face to Face Meeting Encoding through laptop	Completed
11/19/24	Gathering of review of literature	Encoding through laptop	Completed
11/20/24	Revision of Chapter 1	Face to Face meeting Consultation with Research Adviser	Completed

12/1/24	Chapter 1 finalization	Face to Face Meeting Encoding through laptop	Completed
12/1/24	Formulation of grading criteria for chapter 2	Face to Face Meeting Encoding through laptop	Completed
12/2/24	Revision of Chapter 2	Face to Face meeting Consultation with Research Adviser	Completed
12/4/24	Chapter 2 Finalization	Face to Face Meeting Encoding through laptop	Completed
12/5/24	Consultation with the statistician	Face to Face Meeting Encoding through laptop	Completed
12/6/24	Final Revisions of Chapter 1 and Chapter 2	Face to Face Meeting Encoding through laptop	Completed
12/7/24	Re-Consultation with the adviser and statistician	Face to Face Meeting Encoding through laptop	Completed
12/8/24	Preparation of Documents for Proposal Defense	Face to Face Meeting Encoding through laptop	Completed
12/9/24	Final manuscript passed for Defense	Face to Face Meeting	Completed
12/11/24	Title Proposal	Face to Face Meeting	Completed
12/20/24	Final Revisions	Face to Face Meeting Encoding through laptop	Completed
1/7/25	Site Visit in DMMMSU-NLUC and the	Face to Face Meeting	Completed

	Bee Apiaries in Bacnotan, La Union		
1/17-23/25	Submission of Revised Research Paper for endorsement to REC	Face to Face Meeting	Completed
1/27/25	Endorsement and Submission of Research Paper to the REC.	Face to Face Meeting	Completed
1/31/25 – 3/10/25	Preparation for Experimentation and Data Gathering	Face to Face Meeting	Completed
2/11/25	Approval of the Research Paper by the REC	Google Mail	Completed
2/27/25	Accomplishment of Progress Report	Google Mail	Completed
3/3/25	Plant Identification in DMMMSU-NLUC	Face to Face Meeting	Completed
3/10/25	Honey Collection	Face to Face Meeting	Completed
3/12/25	Plant Specimen Collection	Face to Face Meeting	Completed
3/12/25 – 4/9/25	Experimentation and Data Gathering	Face to Face Meeting	Completed
3/12/25	Sanitization of grossing materials	Face to Face Meeting Laboratory Work	Completed
3/12/25	Aloe vera extraction and honey preparation	Face to Face Meeting Laboratory Work	Completed

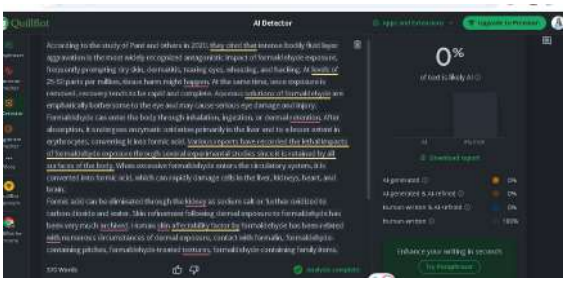
3/12/25	Preparation of treatment samples	Face to Face Meeting Laboratory Work	Completed
3/13/25	Collection of liver, Specimen Grossing, and Fixation Proper	Face to Face Meeting Laboratory Work	Completed
3/13/25	Accomplishment of Progress Report	Google Mail	Completed
3/14/25	Tissue processing (Dehydration, Clearing, and Embedding)	Face to Face Meeting Laboratory Work	Completed
3/15/25	Tissue processing (Section-cutting, Mounting of tissue sections, Staining, and Mounting)	Face to Face Meeting Laboratory Work	Completed
3/27/25	Accomplishment of Progress Report	Google Mail	Completed
4/9/25	Evaluation of Evaluator 1 and 2	Face to Face Meeting Laboratory Work	Completed
4/10/25	Accomplishment of Progress Report	Google Mail	Completed
4/11/25	Evaluation of Evaluator 3 and 4	Face to Face Meeting Laboratory Work	Completed
4/11/25	Evaluation of Evaluator 5	Face to Face Meeting Laboratory Work	Completed

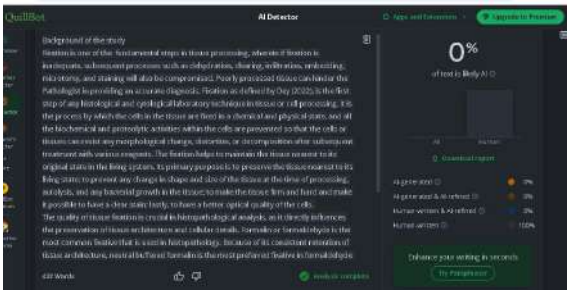
4/11/25	Consultation with the Statistician	Face to Face Meeting Laboratory Work	Completed
4/16/25	Mock Research Defense with the Adviser	Google Meet	Completed
4/10-17/25	Accomplishment of Chapter 3 and 4 Finalization of Whole Research Paper for Final Research Defense	Face to Face Meeting Google Mail	Completed
4/21/25	Final Research Defense	Face to Face Meeting	Completed
4/22/25-4/28/25	Revision of the manuscript	Face to Face Meeting Google Mail	Completed

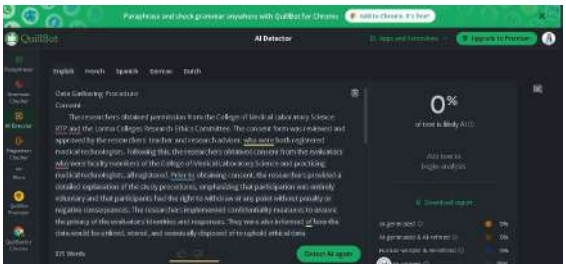
Appendix O

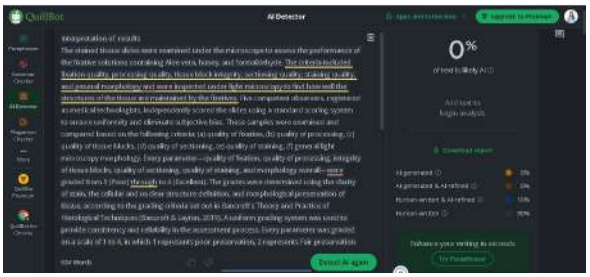
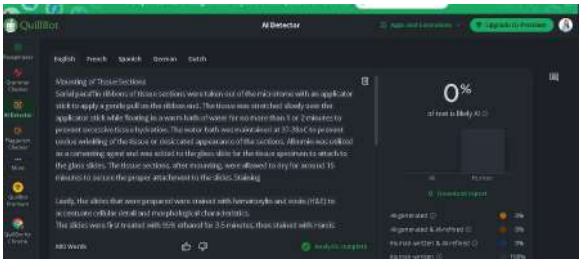
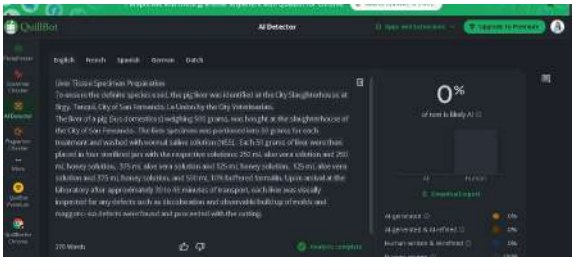
CERTIFICATE OF AI CONTENT ANALYSIS

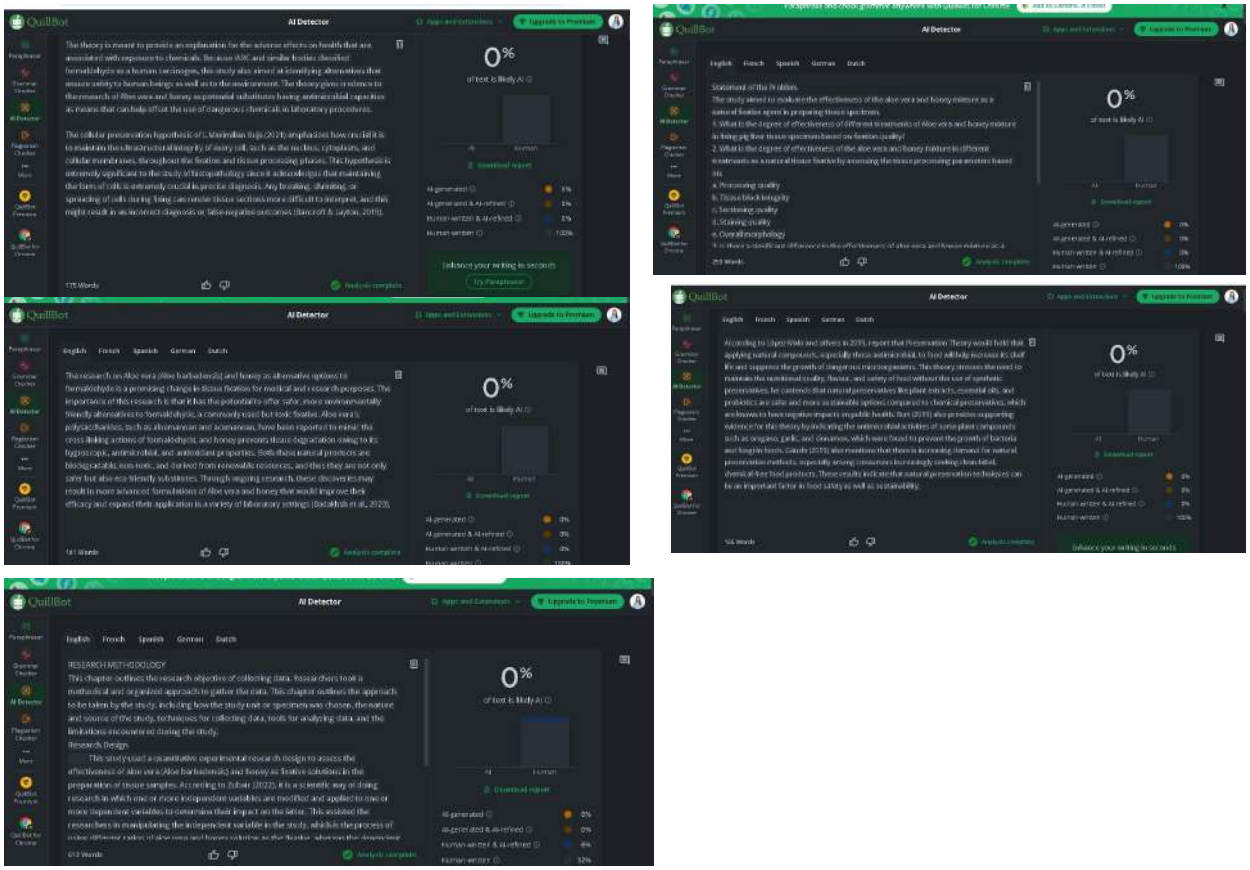
This certificate is to verify that the submitted file, titled "**ALOE VERA *(Aloe barbadensis)* AND HONEY *(Apis mellifera)* AS NATURAL FIXATIVES FOR TISSUE FIXATION IN HISTOPATHOLOGY**" has undergone an analysis for the presence of AI-generated content. Based on the analysis conducted, the submitted text is determined to be:









This indicates that the text is highly likely to be human-written. Issued on the 27th day of April, 2025, in San Fernando, Ilocos Region, Philippines.

Authors Biodata

The researchers are third-year Bachelor of Medical Laboratory Science students at Lorma Colleges. They are academically driven with active participation in research and seminars. With a strong passion for learning and growth, they are committed to contribute in the medical field, becoming competent and dedicated medical technologists in the future.