POMELO (*Citrus maxima)* AS A CLEARING EXTRACT IN TISSUE PROCESSING

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ABSTRACT

Clearing is a critical step in tissue processing which involves removing alcohol from tissue samples to prepare them for paraffin embedding, in a routine tissue processing. Xylene is volatile, readily producing flammable and toxic concentrations at room temperature. A known non-toxic substance with similar characteristics with xylene is called D-limonene. It appears to perform adequately as a wax solvent and cleaning agent, as it has a reduced fire risk compared with xylene. In this study, D-Limonene from pomelo peel extract was utilized as a clearing agent in tissue processing. This study aims to: 1) determine the effect of pomelo peel extract on chicken liver tissue; 2) investigate the most effective clearing time of pomelo peel extract; and 3) compare the effectiveness of different treatments against xylene. The control group consisted of 3 replicates of chicken liver tissue cleared with xylene, while the experimental group included 3 treatments; treatment 1 (60 minutes), treatment 2 (120 minutes), and treatment 3 (180 minutes) with 3 replicates each. Statistical analysis revealed that the mean of treatment 2 indicated a good clearing agent in terms of clearing ability through evaluation of the translucency, evaporation rate in the paraffin oven, compatibility with the hematoxylin and eosin stain, morphological changes of the chicken liver tissue based on the degree of shrinkage or swelling, while the control group showed a satisfactory clearing ability. These results indicate the potential of pomelo (*Citrus maxima*) peel extract as an alternative clearing agent in tissue processing. Therefore, further studies are strongly recommended.

Keywords: *Clearing; D-limonene; Tissue Processing; Xylene; Clearing Agent*

INTRODUCTION

In histopathology, clearing involves removing alcohol from tissue samples to prepare them for paraffin embedding, with xylene serving as a critical solvent. After being dehydrated with increasing concentrations of alcohol, tissues are immersed in xylene, which effectively clears the sample by replacing the alcohol and allowing for better paraffin wax infiltration. This is critical because xylene has a low viscosity and is highly miscible with alcohol and paraffin, allowing the tissue to maintain its structural integrity while becoming compatible with the embedding medium. The significance of xylene lies in its function as a clearing agent and in improving the clarity and quality of histological sections, which is critical for accurate diagnosis and research (Clifton, 2024).

Xylene is an organic compound with the formula $(CH^3)^2C^6H^4$ that exists in three isomeric forms: ortho-xylene, meta-xylene, and para-xylene, each with a different arrangement of methyl groups on a benzene ring. Xylene is an aromatic hydrocarbon and it is widely used as a solvent in a variety of industrial applications, including painting and cleaning. Its cleaning ability is notable for its high solvent power, which allows it to effectively dissolve oils, greases, and other stubborn substances, making it a popular choice for removing tough stains from surfaces. Furthermore, xylene's ability to evaporate quickly increases its effectiveness as a clearer by leaving little residue after use (Toppr, 2020).

Despite its advantages as an excellent clearing agent, xylene is volatile, readily producing flammable and toxic concentrations at room temperature. Its vapor is heavier than air and the accumulated odor generally provides adequate warning of hazardous concentration (Biosolvents, 2022). It also induces toxicity to laboratory personnel and environmental risk. Xylene's hazardous consequences include acute neurotoxicity, heart and kidney injury, cancer, blood dyscrasias, skin illnesses, gastrointestinal disturbances, musculoskeletal system abnormalities, and fetotoxicity among others (Abdollahi and Niaz, (2015)).

The lipophilic property of xylene makes it rapidly absorbed by all routes of exposure, rapidly distributed throughout the body, and, if not metabolized, quickly eliminated in exhaled air (Agency for Toxic Substances and Diseases Registry (ATSDR), 2007). It has been reported the highly toxicity and carcinogenic potential of this clearing agent. The level of exposure depends upon the dose, duration, and work being done.

The use of xylene in a variety of industrial applications, particularly in histopathology for tissue processing and staining, has raised concerns about its toxicity and potential health hazards. Recent studies have highlighted the need to investigate safer solvents; for example, the National Society for Histotechnology (2019) states that "The exploration of natural extracts

as substitutes for xylene is essential not only to mitigate health risks associated with chemical exposure but also to enhance the sustainability of laboratory practices." This statement emphasizes the importance of finding safer alternatives while promoting environmentally friendly methodologies in scientific research.

A known non-toxic substance with similar characteristics with xylene is called D-limonene. D-Limonene, also known as *Citrus terpenes*, is the main chemical constituent found in the cold- compressed peel oils that can be derived from all edible citrus fruits like pomelo with a concentration of 33.61%. The concentration of the D-limonene varies from the citrus variety like the Lemon *(Citrus limon)* with a concentration of 45%-76% and Bergamot orange (*Citrus bergamia*) with 32%-45% (Bora, et.al, 2020). D-Limonene is readily absorbed, metabolized, and cleared from the body. It appears to perform adequately as a wax solvent and cleaning agent, as it has a reduced fire risk compared with xylene (Niaz, et.al, 2015).

The natural acidity of pomelo *(Citrus maxima)* peel, along with its bioactive constituents such as vitamin C, hesperidin, and naringenin, contributes to its ability to remove impurities, dead skin cells, and other residues through its antioxidant activity (Sharma et al., 2024). This experimentation intends to analyze the clearing properties of the pomelo peel extract and its potential in histology concerns. With the many possible benefits of the pomelo peel extract, this work focuses on providing an effective and eco-friendly method of clearing histological samples.

Xylene and D-limonene, are both organic solvents that belong to the class of hydrocarbons: xylene is an aromatic hydrocarbon (LeMay, 2015), while D-limonene is a cyclic terpene (Pagliaro et al., 2023). They are both hydrophobic, meaning they do not mix with water, and are effective in dissolving non-polar substances. These solvents are used in industries such as paint production, adhesives, and cleaning products due to their ability to dissolve oils and other residues effectively (Adhesives and Sealants Industry, 2015).

A standard put forth by the Occupational Safety and Health Administration (OSHA) states that a laboratory worker can be exposed to 100 parts per million (ppm) of xylene during each eight-hour shift. The National Institute for Occupational Safety and Health (NIOSH) puts xylene's Immediately Dangerous to Life or Health (IDLH) air concentration value at 900 ppm, which is essentially the point at which a worker will experience an almost immediate negative health effect (Biotech, 2018). The World Health Organization has emphasized that xylene exposure can cause major health consequences, such as respiratory problems and brain damage, leading to the need for safer substitutes in clinical settings.

This study aimed to assess pomelo peel extract as a safer, cost-effective alternative to xylene in tissue clearing by evaluating its effects on clearing ability, evaporation rate, stain compatibility, and liver tissue morphology. Promoting safer and more sustainable laboratory practices, the findings help students recognize the importance of environmental and health considerations in laboratory work and support future research in improving histological protocols in the MLS field.

Theoretical/Conceptual Framework of the Study

The major theory of the study is Snellius' Refractive Index Matching Theory (1621), as described by Mendoza et al. (2018). This theory involves adjusting the refractive indices of components to visualize internal structures more clearly. It supports tissue clearing, which enhances sample transparency by equalizing refractive indices, allowing light to pass through without scattering, thus improving visualization. This theory is relevant to the study, as it explores the clearing capability of pomelo peel extract. The cellular microenvironment refers to the local surroundings with which cells interact. These microenvironmental cues influence cell behavior, crucial for tissue development and regeneration, including differentiation and growth (Collin et al., 2017). This theory is significant to the study as it examines how the pomelo peel extract affects the cells within the tissue’s microenvironment.

The Endobiogeny Theory (Lapraz & Hedayat, 2013) elaborates on understanding human biology by emphasizing the interrelatedness of bodily systems and the role of the endocrine system in health. It advocates for a holistic clinical approach, incorporating traditional biomedical methods and systems biology. The theory suggests using medicinal plants, such as the pomelo peel extract in this study, when pharmaceutical drugs are unnecessary, supporting the plant-based approach in the research.

The Natural Solvent Compatibility Theory (2020) posits that plant-based extracts, like limonene in pomelo peel, enhance tissue processing. Limonene interacts with lipids and proteins, facilitating better visualization due to improved transparency. Its non-toxic and biodegradable properties make it a safer and more cost-effective option, aligning with environmental and health standards (Zhang et al., 2017; Rafiq et al., 2018; Khan et al., 2020).

A study by Yashaswini and Arvind (2018) explored eco-friendly solvents to replace toxic histology cleaning agents, highlighting the potential of citrus peel phytochemicals. Pomelo peel's antioxidants and solvent properties offer an eco-friendly alternative to synthetic solvents. This research contributes to the knowledge of using citrus bioactive compounds, like limonene, in histology, providing a safer, environmentally friendly substitute for harmful chemicals.

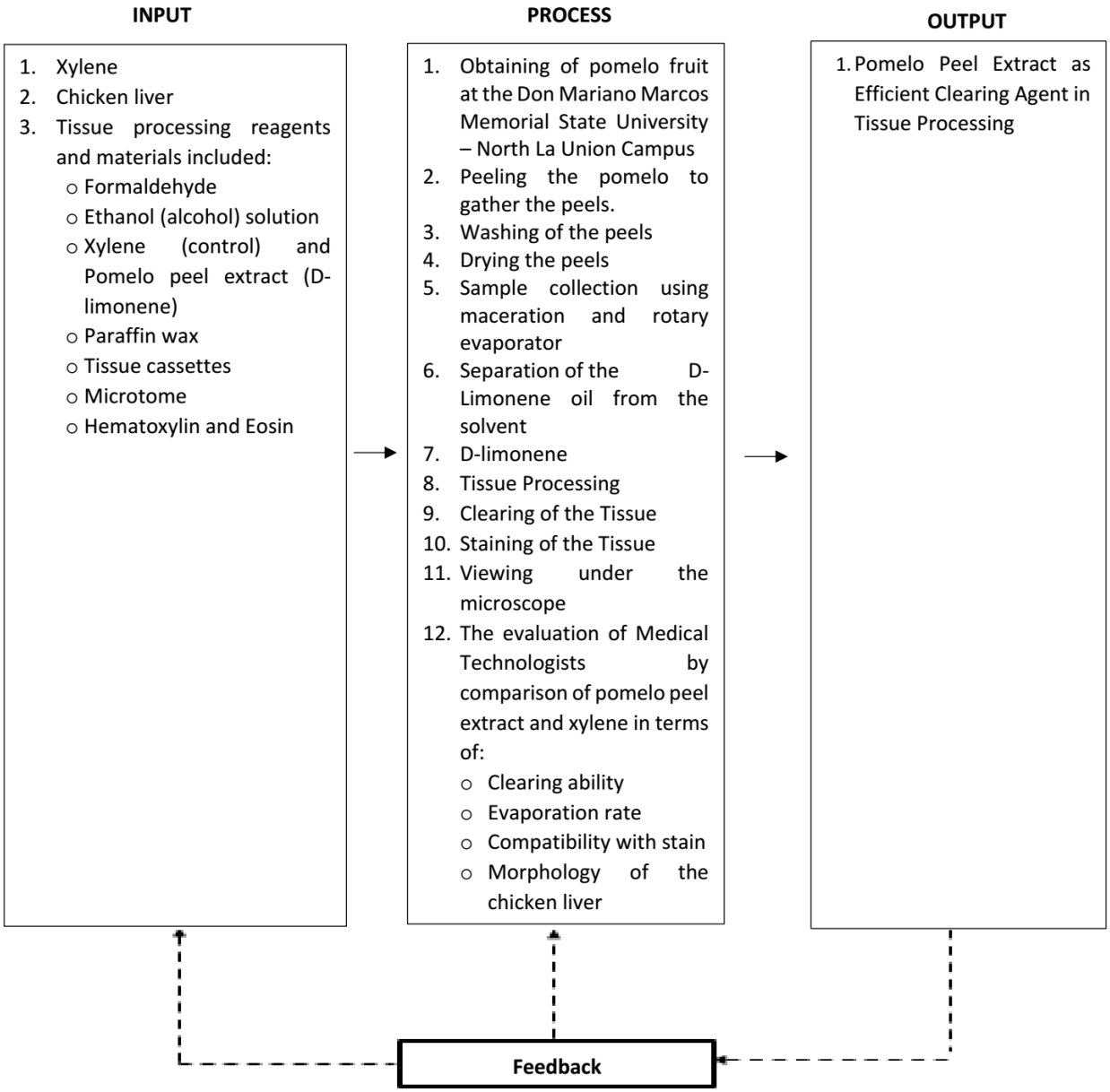

Figure 1: *The Research Paradigm of the Study*

Significance of the Study

This study is significant because it explores pomelo (*Citrus maxima*) peel extract, which contains D-limonene, as a natural and safer alternative to xylene in tissue processing considering its effect on clearing ability, evaporation rate, compatibility with stain and morphology of the chicken liver. This will also minimize the toxic effects and tend to be more economical.

By understanding these substances' effects, students can better appreciate the importance of safety and environmental considerations in laboratory practices. Furthermore,

this research may guide future studies aimed at developing safer options and improving laboratory protocols, ultimately enhancing the quality of education and research in the MLS field.

Objectives of the Study

This study aims to evaluate the effectiveness of pomelo peel extract *(D-limonene*) as a clearing agent in tissue processing. Specifically, this study aims to:

- To determine the effects of different treatments of pomelo peel extract as a clearing agent in tissue processing in terms of:
    - Clearing ability through evaluation of the translucency;
    - Evaporation rate in the paraffin oven;
    - Compatibility with the Hematoxylin and Eosin stain; and
    - Morphological changes of the chicken liver tissue based on the degree of shrinkage or swelling.
- To investigate the most effective time of pomelo peel extract application in clearing tissue samples based on:
    - Clearing ability through evaluation of the translucency;
    - Evaporation rate in the paraffin oven;
    - Compatibility with the Hematoxylin and Eosin stain; and
    - Morphological changes of the chicken liver tissue based on the degree of shrinkage or swelling.
- To compare if there is significant difference between the different treatments of pomelo peel extract and xylene in terms of:
    - Clearing ability through evaluation of the translucency;
    - Evaporation rate in the paraffin oven;
    - Compatibility with the Hematoxylin and Eosin stain; and
    - Morphological changes of the chicken liver tissue based on the degree of shrinkage or swelling.

MATERIALS AND METHOD

This chapter presents the research methodology of the study, selection of study unit or the specimen or population, nature and source or locale of the study, data instrument collection techniques, data statistical analysis tools and limitations of the methodology.

Research Design

This study utilized a quantitative approach through experimental research design. As defined by Singh (2021), an experimental research design is a study that strictly adheres to a scientific research design. It includes the relationship between two (2) variables— the clearing ability of Pomelo (*Citrus maxima*) D-limonene peel extract as the dependent variable and the time of the D-limonene from pomelo peel extract as the independent variables. This was utilized to determine the capability of pomelo peel extract as a clearing agent in chicken liver tissue in which different treatments were observed, tested, and compared statistically, and determine the significant relationship between the dependent and independent variable.

Specimen and Locale of the Study

The study was conducted at the College of Medical Laboratory Science Laboratory, LORMA Colleges, Carlatan, City of San Fernando, La Union. D-limonene was extracted from pomelo peel and used to process 12 liver samples from one broiler chicken (*Gallus gallus domesticus*). Five Registered Medical Technologists (RMTs), all faculty members at LORMA Colleges, evaluated the samples using a grading scale based on four parameters: clearing capability, evaporation rate, staining compatibility, and morphological preservation. All evaluations were performed objectively and confidentially, with no personal data collected. In addition to prior ethical consideration, any bias and discrimination—Fresh passers, Laboratory veterans, or multiple-time board takers are not discriminated against—are heavily avoided nor practiced in the conduct of this research.

To uphold the animal ethical standards in the collection of data, the specimen was treated with moral value regardless of its utility value. The study followed ethical guidelines, ensuring that the research would be postponed or modified if the risks outweighed the benefits. All findings were reported transparently.

Data Gathering Tools

Data was gathered using an observational method through observing the effectiveness of the clearing agent under a microscope, using a compound microscope (Olympus Cx23). Observations were focused on four key parameters: clearing ability through evaluation of the translucency, evaporation rate, compatibility with stain, morphological changes of the chicken liver tissue based on the degree of shrinkage or swelling. These parameters were used to evaluate the overall performance of the clearing agent. A grading scale was gathered from the professionals through conducting evaluation of these key four parameters. Tables and photo documentation was utilized to collect and review the data to validate its accuracy.

Data Gathering Procedure

Consent

The researchers sought permission from the College of Medical Laboratory Science and Lorma College Research Ethics Committee for the performance of the experiment and for the conduct of the evaluation. The consent form was reviewed and approved by the researchers' instructor and research adviser, both of whom are certified medical technologists. When the consent form was approved, the researchers discussed the process of the study, participation, possible unfavorable factors, and benefits of the study to evaluators. This is to inform future medical laboratory science (MLS) students and evaluators about the implications of using D-limonene from pomelo and the negative impacts of xylene exposure. In order to protect their responses and identities, the researchers make sure that all of the evaluators are aware of the confidentiality procedures. They also received information on how the collected data would be used. The evaluators signed a consent form, which acted as a legal agreement confirming their informed and voluntary involvement, to attest to their comprehension and interest in engaging in the evaluation. This procedure is intended to preserve transparency, prevent bias, and support the ethical values of medical technology advancement.

Materials and Equipment

For the collection of pomelo peel extract, beakers, knives, chopping boards, and basins were used, with all glassware thoroughly cleaned to prevent contamination. The pomelo peel was washed with distilled water, sliced, and then underwent maceration and rotary evaporation to obtain the D-limonene. For tissue preparation, knives, chopping boards, basins, and a weighing balance were used. The tissue processing involved a rotary microtome, glass slides (75 mm by 25 mm), a water bath, laboratory stove, paraffin wax, formaldehyde, and alcohol solutions. Hematoxylin and eosin stains were used for staining, while a compound microscope (Olympus CX23) allowed for visualization of the prepared slides.

Pomelo was acquired from Don Mariano Marcos Memorial State University – North La Union Campus, Bacnotan, La Union, maceration which includes sterile bottle for storage and distilled water as a diluent. All equipment for the laboratory assay was provided by the LORMA Colleges, College of Medical Laboratory Science Laboratory.

Equipment Sterilization for Plant Materials Collection and Distillation

All necessary equipment was washed using antibacterial liquid soap and was allowed to air-dry. After drying, all equipment was wiped with 10% hydrogen peroxide cotton swabs and tissue paper and was dried.

Plant Material Collection

Pomelo fruits were acquired from Don Mariano Marcos Memorial State University – North La Union Campus, Bacnotan, La Union. To ensure the use of a single, specific species, the Agriculture Department of Don Mariano Marcos Memorial State University North La Union Campus was consulted to identify the samples. A total of 30 kg of fresh Pomelo peel was collected. The outer layer of the pomelo was separated from the fruit, these peels contain the D-limonene oil needed for the study. After the separation, peels are thoroughly rinsed with water to remove dirt and contaminants that could interfere with the extraction process and dried to reduce moisture.

Preparation of Tissue Liver Specimen

The chicken was slaughtered by the researchers at their boarding house located at Carlatan, San Fernando City 5 minutes away from the College of Medical Laboratory Science Laboratory and was immediately soaked in a 10% formaldehyde to preserve and maintain its viability. The researchers prepared twelve (12) pieces of sample measuring 4 mm by 4 mm with 4 mm thickness coming from one liver only. Each specimen was subjected to their respective processes in their assigned treatments.

Chicken liver was utilized due to its anatomical and physiological similarities to human liver, making it an effective model for studying liver diseases (Auliyah, 2021), its histological structure allows the researchers to observe the cellular and morphological alterations, thus providing valuable insights similar to the human liver. In addition, the ethical considerations surrounding the usage of human liver further support the use of chicken liver in research studies.

D-limonene Collection Process

The pomelo peels were cleaned with distilled water, dried, and processed by removing the pulp and slicing into smaller sections to improve extraction. A total of 6 kilograms of peels were divided into two 7-liter containers, each with 3 kilograms. To each container, 3.2 liters of distilled water were added. The mixtures were sealed and agitated daily for four days under the supervision of Mr. Jerald Macapagal, Laboratory Custodian of the College of Pharmacy. After maceration, the mixtures were filtered through gauze to separate the liquid extract from solid residues. The extract was then subjected to rotary evaporation to yield crude D-limonene.

A rotary evaporator was used to remove solvents from the extract under reduced pressure, concentrating and isolating compounds. The setup included a rotating flask, heated water bath, condenser, and vacuum system. This process yielded 450 mL of crude extract. The extract was filtered and left undisturbed for 48 hours for phase separation. D-limonene oil floated to the top and was collected, yielding 200 mL of isolated oil.

To confirm the presence of D-limonene, a Salkowski test was performed due to the unavailability of High-Performance Liquid Chromatography (HPLC). In this test, 5 mL of the extract was mixed with 2 mL of chloroform and 3 mL of concentrated sulfuric acid. A pale yellow to reddish-brown coloration indicated a positive result for D-limonene (Karthika et al. 2022).

Miscibility Testing

Miscibility refers to the ability of two liquids to form a homogeneous mixture. To test the miscibility of D-limonene with ethanol before tissue processing, three different ratios of D-limonene to ethanol were tested. In test tube 1, 1 mL of D-limonene was mixed with 1 mL of ethanol (1:1). In test tube 2, 2 mL of D-limonene was mixed with 1 mL of ethanol (2:1). In test tube 3, 1 mL of D-limonene was mixed with 2 mL of ethanol (1:2). A completely homogeneous solution indicated good miscibility, suggesting effective transition during tissue processing.

In histological tissue processing, ethanol was used to remove water in dehydration, followed by clearing agents like xylene or D-limonene for alcohol replacement, enabling proper paraffin infiltration. If dehydrating and clearing agents are immiscible, incomplete clearing can occur, leading to poor paraffin infiltration, tissue artifacts, uneven staining, and tissue distortion. Proper miscibility ensures a smooth transition between steps, allowing the clearing agent to effectively replace the dehydrant, ensuring proper tissue clearing, and preserving the tissue's morphology, structural integrity, and staining quality.

Tissue Processing

The process of tissue processing begins with fixation, where liver samples were immersed in a fixative solution to maintain their structural integrity and chemical composition. Following this, dehydration was carried out by immersing the samples in a series of ethanol solutions with increasing concentrations. Next, in the clearing step, xylene was utilized for the control group, while d-limonene, derived from pomelo peels, was employed for the treatment groups. For infiltration, the specimens, contained within cassettes, undergo repeated immersions in molten paraffin wax at 60°C for a total duration of one hour to ensure thorough saturation. After infiltration, the specimens were allowed to cool down to room temperature. Subsequently, embedding involves enclosing the cleared tissue within molten paraffin. The solidified paraffin block containing the tissue was then subjected to sectioning, where a microtome was used to cut it into very thin slices. These paraffinized tissue ribbons were carefully handled during mounting; they were removed from the microtome using an applicator stick, gently stretched over the blade in a 30°C water bath to prevent wrinkles, and then adhered to glass slides using egg albumin as a bonding agent before drying. To visualize the cellular

structures, the sections undergo staining with Hematoxylin and Eosin. Finally, labelling was performed to ensure proper specimen identification by marking the slides according to their respective treatments and group replicates.

Preparation of Treatment Samples

With the modifications to the treatment preparations of Bright et al. (2024), the chicken liver tissue was cleared using 100% D-limonene in varying schedules, 60 minutes for treatment 1, 120 minutes for treatment 2 and 180 minutes for treatment 3. The tissues were also cleared using xylene for 2 changes, 1 hour each change as the positive control. Each treatment has three replicates. The different treatments are presented in Table 1.

Table 1. *Treatment Distribution*

Treatments	Constituents	Time of Clearing (Minutes)
T+	200 mL Xylene	120 Minutes
T1	200 mL of 100% D-limonene	60 Minutes
T2	200 mL of 100% D-limonene	120 Minutes
T3	200 mL of 100% D-limonene	180 Minutes

RESULTS AND DISCUSSION

Table 1: *Effects of the Different Treatments of Pomelo Peel Extract in Terms of Clearing Ability, Evaporation Rate, Compatibility with the H&E Stain, and Morphological Changes Based on the Swelling and Shrinkage*

Treatments	Clearing Ability		Evaporation Rate		Compatibility		Morphological changes	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
T+	**2.600**	**0.910**	**2.333**	**0.816**	**2.200**	**0.941**	**2.600**	**0.986**
T1	**2.666**	**0.723**	**2.333**	**0.617**	**2.400**	**0.632**	**2.733**	**0.961**
T2	**3.400**	**0.507**	**3.000**	**0.756**	**3.266**	**0.704**	**3.333**	**0.488**
T3	**2.200**	**0.775**	**1.466**	**0.743**	**2.000**	**0.926**	**2.067**	**0.884**

The study found that pomelo peel extract, particularly at 120 minutes of exposure,

showed the best overall performance in clearing ability, evaporation rate, stain compatibility, and preservation of tissue structure. Although it produced clear and well-preserved tissues, it showed reduced uptake of hematoxylin, leading to weak nuclear staining. In comparison, xylene consistently provided reliable results across all parameters, maintaining good translucency, stain quality, and structural integrity. Shorter exposure to pomelo extract gave results similar to xylene but with minor inconsistencies and minimal morphological changes, while prolonged exposure led to poor clearing and tissue damage. However, despite treatment 2 showing the highest statistical mean in the demonstration of tissue translucency, evaporation rate, stain compatibility, and tissue morphology preservation, further evaluations found that the overall performance of the positive control (T+) using xylene still yielded the most consistent and reliable results in terms of tissue integrity, clarity, and processing efficiency, reinforcing its current standard use in tissue processing.

CONCLUSION

Based on above findings, pomelo peel extract showed a strong clearing ability on chicken liver tissue. Tissue treated with the extract became translucent, allowing easy observation of cellular details. The 120-minute treatment preserved tissue morphology best, while longer exposure caused distortion. The pomelo peel extract proved effective as a natural clearing agent.

The most effective clearing time for pomelo peel extract was Treatment 2 (120 minutes). At this duration, tissue samples displayed optimal translucency, compatible with hematoxylin and eosin stain, and tissue perfectly preserved, with structures appearing intact and undistorted. Treatment 1 (60 minutes) resulted in moderate clearing, while Treatment 3 (180 minutes) exposures led to tissue damage.

At a significance level of 0.01, most of the result was no significant difference between xylene and any of the three pomelo peel extract treatments (60, 120, and 180 minutes). However, at a p-value of 0.05, some differences became significant, particularly in evaporation rate, stain compatibility, and tissue morphology. Among the treatments, the 120-minute duration showed the closest and most comparable results to xylene, while the 60-minute and 180-minute treatments were generally less effective.

Based on the findings of the study, it is recommended that future research should explore the application of pomelo peel extract on different types of tissues and assess its compatibility with a broader range of staining techniques beyond Hematoxylin and Eosin. It is also advised to compare the extract with other citrus fruits that are rich in D-limonene, to determine whether similar or improved clearing efficiency can be achieved. For better

statistical accuracy and reliability, larger sample sizes should be used in future experiments. Furthermore, implementing blind evaluations by assessors can help reduce potential bias in grading tissue samples

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APPENDICES

Appendix A

Letters of Intent to Conduct a Study

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

October 3, 2024

JOSEPHINE V. CULATON-MILAN, MSMT, RMT
Dean of CMLS
LORMA College of Medical Laboratory Science
Center for Health Sciences - Carlatan Campus
City of San Fernando, La Union 2500

RE: LETTER OF INTENT TO CONDUCT A STUDY

Warm greetings of peace and health!

The undersigned BMLS third year students are interested to conduct a descriptive cross-sectional research entitled "Orange (Citrus Reticulata) Peel Extract As A Clearing Agent In Tissue Processing". This is in partial fulfillment of the requirements for the course MRESEARCH1: Introduction to Medical Laboratory Science Research 1.

On this regard, we respectfully request your acceptance and approval regarding the conduct of our research study.

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

Respectfully yours,
The researchers of BMLS III - Section 2 Group 5

JHOANA MARIE F. ALAGNA
JOHN PHILIP V. ALMODOVAR
JHELL ASHLEY J. CALUB
YVONNE G. LAGARTEJA
JUNE JOSHUA R. MAGLALANG
RAZEN B. MERCADO
HEENA R. SHEETAK

Noted By:

JOSE ENRICO SUMAYA
MLS Research 1 Instructor

BRYLLE KEVIN UGAY
Research Faculty Adviser

Recommending Approval:

MARK ERICSON B. BALADAD, MMPHA, RMT
Research Coordinator, LORMA College of Medical Laboratory Science

Approved by:

JOSEPHINE V. CULATON-MILAN, MSMT, RMT
Chair, Lorma College of Medical Laboratory Science

APPENDIX B

Letter of Intent to the Faculty Research Adviser

LORMA COLLEGES
Your Pathway to a Brighter Future

LORMA COLLEGE OF MEDICAL LABORATORY SCIENCE
Carlatan, City of San Fernando, La Union, Philippines, 2500

October 3, 2024

BRYLLE KEVIN 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 an experimental research entitled **"Exploring Orange Peel Extract as a Non-Toxic Clearing Agent in Histology Pathology"**. This is in partial fulfillment of the requirements for the course MRESEARCH1: Introduction to Medical Laboratory Science Research 1.

In this regard, we are humbly requesting 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 research, titled exploring **"Orange Peel Extract as a Non-Toxic Clearing Agent in Histopathology",** aims to explore the clearing properties of D-Limonene from orange peel extract as a safer alternative to xylene. We intend to compare tissue specimens processed with orange peel extract against those processed with xylene, focusing on tissue morphology and transparency. Given your expertise in this field, We would greatly appreciate your insights on my research methods and experimental design. Your guidance will be invaluable in ensuring the success of this study.

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

Name: Yvonne G. Lagarteja (BMLS III-2)
Phone number: 09470168700
E-mail: *yvonne.lagarteja@lorma.edu*

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

Thank you very much and God bless!

Respectfully yours,

Almodovar, John Philip V.
Maglalang, June Joshua R.
Alagna, Jhoana Marie F.
Caleb, Jhell Ashley J.
Lagarteja, Yvonne G.
Mercado, Razen B.
Sheetak, Heena R.

Student Researchers

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

Conformed:

Brylle Kevin Ugay
Faculty Research Adviser Date:

Appendix C

Contract of Acceptance for Faculty Adviser

LORMA COLLEGES
Your Pathway to a Brighter Future

LORMA COLLEGE OF MEDICAL LABORATORY SCIENCE
Carlatan, City of San Fernando, La Union, Philippines, 2500

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:**"Exploring Orange Peel Extract as a Non-Toxic Clearing Agent in Histology Pathology".**

Conformed:

Brylle Kevin Ugay
Faculty Research Adviser Date:

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 D

Application for Review

LC-REC Form #010
APPLICATION FOR REVIEW FORM

LORMA COLLEGES

APPLICATION FOR REVIEW

(Adapted from National Ethics Guidelines for Health and Health-Related Research 2017)

INSTRUCTION: Please accomplish the form and ensure that all necessary documents are included in your submission.

I. GENERAL INFORMATION:

Title of the Study: Pomelo (*Citrus maxima*) Peel Extract as a Clearing Agent in Tissue Processing

REC Code : _____ No. of Study Participants: : _____N/A_____

Study Site : LORMA Colleges, Carlatan, City of San Fernando, La Union

Name of Researcher/s: Alagna, Jhoanna Marie F., Almodovar, John Philip V., Calub, Jhell Ashley J., Lagarteja, Yvonne G., Maglalang, June Joshua R., Mercado, Razen B., Sheetak, Heena R.

Contact Information : Telephone Number: _____ Mobile Number: 09470168700

Fax Number: _____ Email : yvonne.lagarteja@lorma.edu

Name of Institution: Lorma Colleges of Medical Laboratory Science

Institution's Address : Carlatan, San Fernando City, La Union

Type of Study: Sponsored Clinical Trial ☐Biomedical Research
☐Researcher-Initiated Clinical Trials ☐Stem Cell Research
☐Health Operations Research ☐Genetic Research
☐Social or Behavioral Research ☐Others: Experimental Research
☐Public Health or Epidemiologic

Source of Funding : ☐Self-Funded ☐Scholarship/Research Grant
☐Government-Funded ☐Institution-Funded
☐Sponsored by Pharmaceutical Company
☐Others: _____

Duration of the Study: Start Date: August 2024 End Date: _____May 2025_____

Has the Research Undergone Technical Review? ☐Yes ☐No
(Please attach Technical Review Result)

Has the Research been Submitted to Another Research Ethics Committee? ☐Yes ☐No

II. BRIEF DESCRIPTION OF THE STUDY (Use Extra Sheet if Necessary)

_____The aim of this study is to compare the efficacy of pomelo peel extract, as a supplementary option for xylene in hematoxylin and eosin staining procedure considering its effect on clearing capability, compatibility with stain, tissue morphology and its miscibility with ethanol. This will also minimize the toxic effects and tend to be more economical. This is to inform future medical laboratory science (MLS) students

and researchers about the implications of using pomelo peel extract and the negative impacts of xylene exposure.

III. CHECKLIST OF DOCUMENTS FOR SUBMISSION

a. Basic Requirements

☐Letter of Intent to Conduct a Study ☐Full Proposal/Study Protocol

☐Filled-up Application Form for Review ☐Budget

☐Endorsement of the RTP ☐Funding Institution

☐Timetable ☐Curriculum Vitae of Researcher

b. Supplementary Documents (if applicable)

☐Questionnaire ☐Philippine FDA Marketing Authorization or

☐Data Collection Forms Import Licensure

☐Product Brochure ☐Permit/s for Special Population

☐Others: _____

Accomplished by: _____ Date Submitted: _____

(Signature over Printed Name)

(to be filled-out by the Secretariat)

Completeness of Documents: ☐Complete ☐Incomplete

Remarks:

_____

_____

_____

Date Received: _____ Received by: _____

January 22, 2025

MR. RYAN JAY G. MOSTOLES, MASE, RMT, CT
Chair, LORMA Colleges – Research Ethics Committee
LORMA Colleges, Inc.
Carlatan, City of San Fernando, La Union

SUBJECT: Application for Ethics Review by the LC-REC

Greetings with a LORMA smile!

The undersigned Third Year BS MLS students at the LORMA College of Medical Laboratory Science intends to conduct a study entitled: "ORANGE (*Citrus reticulata*) PEEL EXTRACT AS A CLEARING AGENT IN TISSUE PROCESSING".

This study aims to determine the efficacy of orange peel extract as a clearing agent in tissue processing. The result of this study will give insights into the efficacy of orange peel extract, as a supplementary option for xylene in hematoxylin and eosin staining procedure considering its effect on clearing capability, compatibility with stain, tissue morphology and its miscibility with ethanol. This will also minimize the toxic effects and tend to be more economical.

In light of this, the student researchers would want to apply for ethical review by the LORMA Colleges – Research Ethics Committee.

Your kind approval and acceptance would be invaluable for the conduct of this study. Should there be any questions, please feel free to contact the lead proponent directly.

Name: Yvonne G. Lagarteja
E-mail: yvonne.lagarteja@lorma.edu
Mobile number: 09470168700

We are respectfully anticipating a favourable reply from you. Advanced thank you for considering our application and we pray that God may continue blessing you and your loving family!

Sincerely,

JHOANA MARIE F. ALAGNA
JHELL ASHLEY J. CALUB
JUNE JOSHUA R. MAGLALANG
JOHN PHILIP V. ALMODOVAR
YVONNE G. LAGARTEJA
RAZEN B. MERCADO

HEENA R. SHEETAK
Student Researchers, College of Medical Laboratory Science

Noted by:

BRYLLE KEVIN UGAY, RMT
Faculty Research Adviser

MARK ERICSON B. BALADAD, MMPHA, RMT
Research Coordinator, LORMA College of Medical Laboratory Science

1/21/25
JOSEPHINE V. CULATON-MILAN, MSMT, RMT
Dean, LORMA College of Medical Laboratory Science

Appendix E

Informed Consent Form

LORMA COLLEGES

INFORMED CONSENT FORM

INSTRUCTION: Please accomplish the form and ensure that all necessary documents are included in your submission.

GENERAL INFORMATION:

Title of the Study: Pomelo (*Citrus maxima)* Peel Extract as a Clearing Agent in Tissue Processing

REC Code : _____ No. of Study Participants: N/A

Study Site : LORMA Colleges, Carlatan, City of San Fernando, La Union

Name of Researcher/s: Jhoanna Marie F. Alagna, John Philip V. Almodovar, Jhell Ashley J. Calub, Yvonne G. Lagarteja, June Joshua R. Maglalang, Razen B. Mercado, Heena R. Sheetak

Contact Information : Telephone Number: _____ Mobile Number: 09470168700

Fax Number: _____ Email : yvonne.lagarteja@lorma.edu

Name of Institution: LORMA Colleges of Medical Laboratory Science

Institution's Address : Carlatan, City of San Fernando, La Union

Type of Study: □ Sponsored Clinical Trial □ Research
□ Researcher-Initiated Clinical Trials □ Stem Cell Research
□ Health Operations Research □ Genetic Research
□ Social or Behavioral Research □ Others: Experimental Research
□ Public Health or Epidemiologic

Source of Funding : ☑ Self-Funded □ Scholarship/Research Grant
□ Government-Funded □ Institution-Funded
□ Sponsored by Pharmaceutical Company
□ Others: _____

Duration of the Study: Start Date: August, 2024 End Date: May, 2025

INTRODUCTION (Use Extra Sheet if Necessary)

This paper describes the investigation of D-limonene obtained from pomelo peels (*Citrus maxima*) and its potential use as a clearing solvent for tissue samples. It analyses the degree of clearing D-limonene achieves, its evaporation rate, its compatibility with different stains, and its impact on the morphology of the tissues. The investigation seeks an answer by using natural and benign processes while addressing the problems with existing clearing solvents, frequently used in most histological applications.

PURPOSE OF RESEARCH (Use Extra Sheet if Necessary)

This study intends to determine the usefulness of D-limonene from pomelo peels as a non-toxic clearing agent in histology. In particular, it aims to test the effectiveness of D-limonene in relation to its opacity, evaporating potential, interaction with stains, and tissue morphology to develop friendly and economically viable means of tissue clearing.

TYPE OF RESEARCH INTERVENTION

1. Participant Selection

We do not aim to test a certain tumor morphology, and the participants are selected through convenience sampling. We will not include participants who refuse to participate in the grading of tissue slides or those who will not sign the informed consent as well as participants who will withdraw from taking any parts of the study.

2. Voluntary Participation

In accordance with the principle of respect for a person, potential participants for the study will not be coerced or pressured in any way to participate in the data gathering procedure. Voluntary participation will be employed by the researchers to recruit any participants for the grading and informed consents will be given. The researchers would not bring tissue samples to examine without the consent and signature of the participants on the letter.

3. Procedures

We will give tissue samples to be graded based on the our provided grading sheet. This research involves evaluation by the MLS (Medical Laboratory Science) faculty, who will rate the clearing ability of the tissue specimens. -(You will receive tissue samples to be graded based on the provided grading sheet. As part of this research, the MLS (Medical Laboratory Science) faculty will evaluate the clearing ability of the tissue specimens, and your expertise and input will be integral to the study's success.)

4. Risks

No known risk are present in the participation of the research

5. Benefits

By participating in this study, you will have the opportunity to be part of research exploring sustainable alternatives in histological practices, allowing you to enhance your knowledge and skills in evaluating clearing techniques. Your involvement may also inspire further research in your field and provide recognition for your expertise in supporting student-led scientific studies.

6. Reimbursements

Reimbursements are not applicable, as this study requires participants to grade tissue samples provided by the researchers.

7. Confidentiality

The feedback you provide as MLS faculty will be treated with the utmost confidentiality and used solely for the purpose of assessing the study's outcomes.

8. Sharing of Results

The results of this study will be reported in oral presentation form in academic conferences, class presentations, or in student research journals, if appropriate.

9. Right to Refuse or Withdrawal

Your participation in this research, including the evaluation you will conduct as MLS faculty, is entirely voluntary. You have the right to decline participation or withdraw at any stage without any repercussions. Your decision will be respected, and any data or ratings you provide can be retracted upon your request, ensuring your autonomy throughout the research process.

10. Who to Contact

Yvonne G. Lagarteja, BMLS III-2 LORMA Colleges

Phone Number: 09470168700

Email: yvonne.lagarteja@lorma.edu

CERTIFICATE OF CONSENT:

I have read the information stated herein or it was properly explained to me. I was provided with a chance to ask questions relative to it. All questions I asked were answered properly; therefore, I consent and voluntarily participate in this study.

Name of Participant: _____

Signature of Participant: _____

Date: _____

Statement from the Researcher/Person Obtaining the Consent

All information pertaining to this study was explained to the possible participant and made sure that he/she fully understood what she/he has to do in the research.

Similarly, I affirm that the potential participant was given with a chance to ask questions which I have answered accurately to the best of my ability.

Likewise, I affirm that the participant was not coerced or forced in giving consent. That he/she has voluntarily provided the consent.

Accomplished by: _____ Date Submitted: _____

(Signature over Printed Name)

Appendix F

Letter of REC approval

LORMA COLLEGES

LC-REC Form #040
CERTIFICATE OF EXEMPTION FROM REVIEW

CERTIFICATION OF EXEMPTION FROM REVIEW

To: Jhoana Marie Alagna, John Philip Almodovar, Jhell Ashley Calub, Yvonne Lagarteja, June Joshua Maglalang, Razen Mercado and Heena Sheetak

From: LORMA Colleges - Research Ethics Committee

Date: January 30, 2025

This is to certify that the Research Proposal entitled, "POMELO *(CITRUS MAXIMA)* PEEL EXTRACT AS A CLEARING AGENT IN TISSUE PROCESSING" submitted by Jhoana Marie Alagna, John Philip Almodovar, Jhell Ashley Calub, Yvonne Lagarteja, June Joshua Maglalang, Razen Mercado and Heena Sheetak of the College of Medical Laboratory Science has been reviewed by the Research Ethics Committee of LORMA Colleges and found that all ethical considerations are in place to conduct the research in the stated locale of the study. Hence, this research proposal is exempted from review.

BEVERLY B. BARUT, RPh
Member, LC-REC

Appendix G

Comments From the REC

LORMA
COLLEGES

CAMPUS FOR HEALTH SCIENCES
GRADUATE STUDIES AND RESEARCH INSTITUTE

ENDORSEMENT

27 January 2025

To the LC-REC:

The research protocol of ***Ms. Alagna Jhoana Matie et al*** entitled, ***"Pomelo Peel Extract as a Cleaning Agent in Tissue Processing"*** is hereby endorsed for Ethics Review ***provided the Research Paradigm will be particularly in the output box. Remove the items which are not considered outputs. Please see page 8 of the manuscript.***

Thank you very much.

Sincerely,

MARITES C. PAGDILAO, MAN, MPA
Chairman, CHS-GSRI

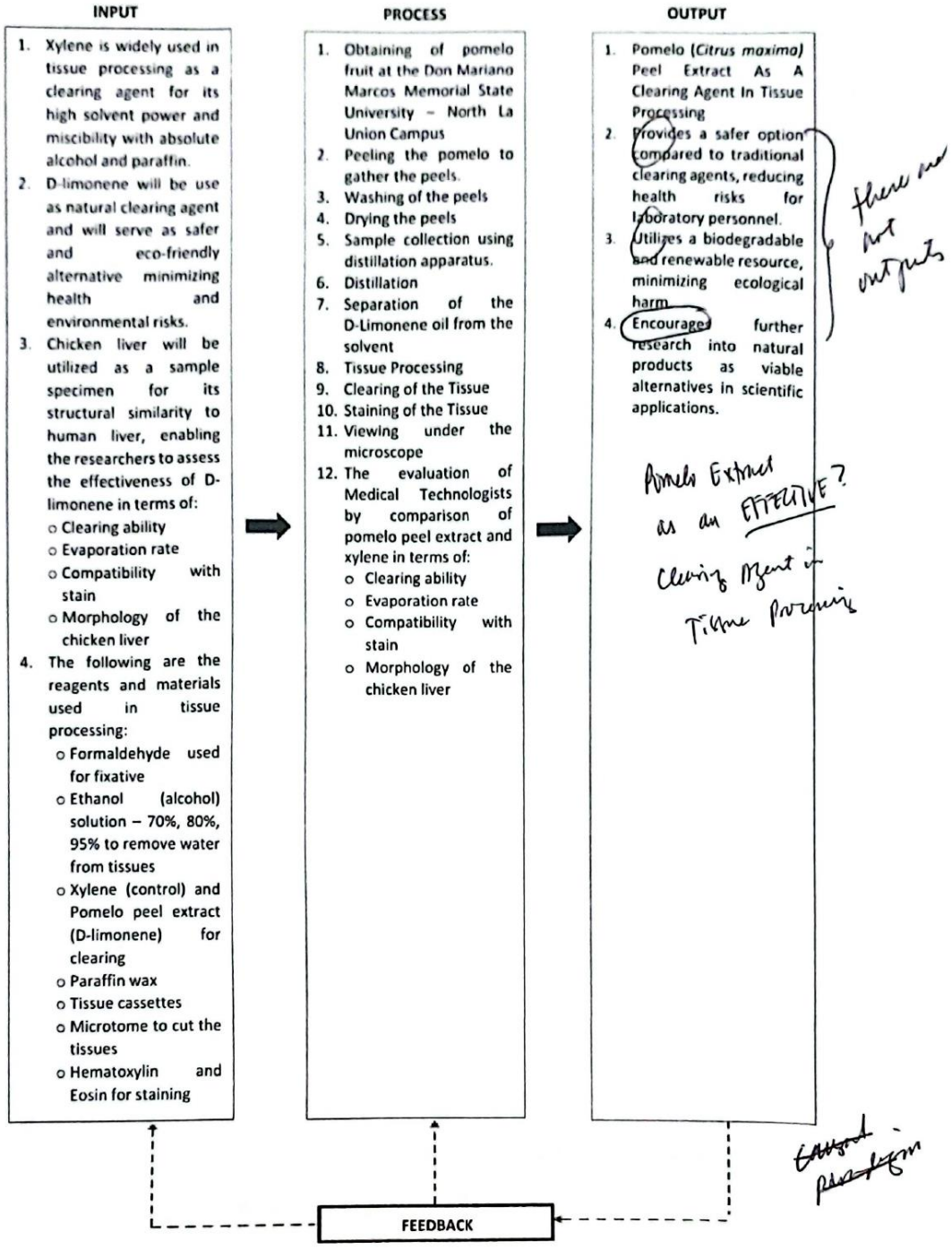

FIGURE 1: The Research Paradigm of the Study

Appendix H

Letter for Plant Identification

LORMA COLLEGES
Your Pathway to a Brighter Future

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

February 27 2025

Dr. Junifer Rey E. Tabafunda
Chancellor
DON MARIANO MARCOS MEMORIAL STATE UNIVERSITY – NORTH LA UNION CAMPUS
Sapilang, Bacnotan, La Union 2515
Philippines

Email: chancellor.nluc@dmmmsu.edu.ph
Phone: **(072) 687-0634 | +63 926 900 4670**

RECORDS OFFICE
DMMMSU – NLUC
RECEIVED
DATE: 02/27/2025
BY: VSI #01-127

Re: Letter to the Chancellor

Warm greetings of peace and health!

02/27/2025

The following BMLS third year students will conduct an experimental research entitled **"Pomelo(*Citrus maxima*) peel extract as a Clearing Agent in Tissue Processing".** This is in fulfillment of the requirements for the course MRESEARCH2: Medical Laboratory Science Research Paper Writing and Presentation.

Our research aims to investigate the feasibility of using Pomelo (*Citrus maxima*) peel extract as a clearing agent in tissue processing.

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

We are beyond grateful for your help and thank you for considering our request, sir.

Should there be any further questions, concerns, or clarifications, please do not hesitate to reach us through our research leader.

Name: Yvonne G. Lagarteja(BMLS III-2)
Phone number: 09470168700
E-mail: yvonne.lagarteja@lorma.edu

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

Respectfully yours,

JHOANA F. ALAGNA. JHELL ASHLEY J. CALUB. JUNE JOSHUA R. MAGLALANG

JOHN PHILIP V. ALMODOVAR. YVONNE G. LAGARTEJA RAZEN B. MERCADO

HEENA R. SHEETAK

Noted by:

FOR. JM Contado
Please extend our assistance to the researchers.
for + in behalf of the Chancellor

Brylle Kevin Ugay, RMT
Faculty Research Adviser

Jose Enrico M. Sumaya, RMT
Instructor, Research Lecture

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 I

Certificate of Plant Identification

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

IDENTIFICATION CERTIFICATE OF PLANT MATERIAL

This is to certify that **JHOANA F. ALAGNA, JHELL ASHLEY J. CALUB, JUNE JOSHUA R. MAGLALANG, JOHN PHILIP V. ALMODOVAR, YVONNE G. LAGARTEJA, RAZEN B. MERCADO AND HEENA R. SHEETAK,** 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 - Lukban
Scientific Name - *Citrus maxima* (Burm.) Merr.
Family Name - Rutaceae

This certification is issued to **Jhoana F. Alagna, Jhell Ashley J. Calub, June Joshua R. Maglalang, John Philip V. Almodovar, Yvonne G. Lagarteja, Razen B. Mercado** and **Heena R. Sheetak** for all legal intentions and purposes.

Issued this 3rd 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 J

Letter to the College of Pharmacy for Rotary Evaporation and Guidance of the Laboratory

Custodian

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

March 7, 2025

Ellen Mae P. Abiqui, RPh, MSPharm, CPT
Executive Director, Campus for Health Sciences & Dean, College of Pharmacy
LORMA COLLEGES
Carlatan, City of San Fernando, La Union
Philippines

Warm greetings of peace and health!

The following BMLS third year students will conduct an experimental research entitled **"Pomelo(*Citrus maxima*) peel extract as a Clearing Agent in Tissue Processing".** This is in fulfillment of the requirements for the course MRESEARCH2: Medical Laboratory Science Research Paper Writing and Presentation.

Our research aims to investigate the feasibility of using Pomelo (*Citrus maxima*) peel extract as a clearing agent in tissue processing.

In this regard, we are humbly requesting for the apparatuses to be used in the rotary evaporator and for the supervision of Mr. Jerald Macapagal during the distillation process of the Pomelo (*Citrus maxima*) peel using the Rotary evaporator

We are beyond grateful for your help and thank you for considering our request, Ma'am.

Should there be any further questions, concerns, or clarifications, please do not hesitate to reach us through our research leader.

Name: Yvonne G. Lagarteja(BMLS III-2)
Phone number: 09470168700
E-mail: yvonne.lagarteja@lorma.edu

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

Respectfully yours,

JHOANA F. ALAGNA. JHELL ASHLEY J. CALUB. JUNE JOSHUA R. MAGLALANG

JOHN PHILIP V. ALMODOVAR. YVONNE G. LAGARTEJA RAZEN B. MERCADO

HEENA R. SHEETAK

Student Researchers

Noted by:

Brylle Kevin Ugay, RMT
Faculty Research Adviser

> 1 cycle only
> 1:00 PM - 6:30 PM (5.5 hours)
March 15, 2025

Jose Enrico M. Sumaya, RMT
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 5/7/25
Dean, LORMA College of Medical Laboratory Science

Appendix K

Letter to Evaluators

LORMA COLLEGES
Your Pathway to a Brighter Future

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

March , 2025

Mr. Raeuelle Ken Novero, RMT
Medical Laboratory Scientist Instructor
Lorma Colleges
Carlatan, San Fernando City, La Union 2500

Subject: Request for Evaluation in Our Research Study

Warm greetings of peace and health!

The undersigned BMLS third-year students will conduct an experimental research study entitled "Pomelo(Citrus maxima) peel extract as a Clearing Agent in Tissue Processing" This study is in fulfillment of the requirements for the course MRESEARCH2: Medical Laboratory Science Research Paper Writing and Presentation.

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a. Clearing ability through evaluation of the translucency.
b. Evaporation rate in the water bath
c. Compatibility with the Hematoxylin and Eosin stain
d. Morphological changes of the chicken liver tissue based on the degree of shrinkage or swelling

Each parameter will be independently assessed using a standardized grading system to ensure consistency and minimize subjective bias.

We sincerely hope for your positive response and support in this research endeavor. Your expertise will greatly contribute to the credibility and reliability of our findings. Should you have any questions or require further details, please do not hesitate to contact us.

Thank you for your time and consideration. We look forward to your favorable response.

Name: Yvonne G. Lagarteja(BMLS III-2)
Phone number: 09470168700
E-mail: yvonne.lagarteja@lorma.edu

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

Respectfully yours,

JHOANA MARIE F. ALAGNA. JHELL ASHLEY J. CALUB. JUNE JOSHUA R. MAGLALANG

JOHN PHILIP V. ALMODOVAR. YVONNE G. LAGARTEJA RAZEN B. MERCADO

HEENA B. SHEETAK

Student Researchers

Noted by:

Brylle Kevin Ugay, RMT
Faculty Research Adviser

Conformed by:

Raeuelle Ken Novero, RMT
Evaluator

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

March 28, 2025

Ms. Hazel Bening, RMT
Medical Laboratory Scientist Instructor
Lorma Colleges
Carlatan, San Fernando City, La Union 2500

Subject: Request for Evaluation in Our Research Study

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HEENA R. SHEETAK

Student Researchers

Noted by:

Brylle Kevin Ugay, RMT
Faculty Research Adviser

Conformed by:

04/07/25
Hazel Bening, RMT
Evaluator

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

March 28, 2025

Mr. Aldrin Patrick Estocapio , RMT
Medical Laboratory Scientist Instructor
Lorma Colleges
Carlatan, San Fernando City, La Union 2500

Subject: Request for Evaluation in Our Research Study

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JOHN PHILIP V. ALMODOVAR. YVONNE G. LAGARTEJA RAZEN B. MERCADO

HEENA R. SHEETAK

Student Researchers

Noted by:

Brylle Kevin Ugay, RMT
Faculty Research Adviser

Conformed by:

Aldrin Patrick Estocapio, RMT
Evaluator

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

March , 2025

Mr. Ponciano Arnobit,RMT
Medical Laboratory Scientist Instructor
Lorma Colleges
Carlatan, San Fernando City, La Union 2500

Subject: Request for Evaluation in Our Research Study

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HEENA R. SHEETAK
Student Researchers

Noted by:
Brylle Kevin Ugay, RMT
Faculty Research Adviser

Conformed by:
Ponciano Arnobit, RMT
Evaluator

LORMA COLLEGE OF MEDICAL LABORATORY SCIENCE
Carlatan, City of San Fernando, La Union, Philippines, 2500
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March 28, 2025

Mrs. Wyndel Catli-Budchangan, RMT
Medical Laboratory Scientist Instructor
Lorma Colleges
Carlatan, San Fernando City, La Union 2500

Subject: Request for Evaluation in Our Research Study

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JOHN PHILIP V. ALMODOVAR. YVONNE G. LAGARTEJA RAZEN B. MERCADO

HEENA R. SHEETAK

Student Researchers

Noted by:

Brylle Kevin Ulay, RMT
Faculty Research Adviser

Conformed by:

4-21-25

Wyndel Catli-Budchangan, RMT
Evaluator

Appendix L

Request Letter for Laboratory Materials

LORMA COLLEGES
Your Pathway to a Brighter Future

LORMA COLLEGES
Carlatan, City of San Fernando
La Union, 2500 Philippines
COLLEGE OF MEDICAL LABORATORY SCIENCE

RESEARCH 2 REQUEST FOR LABORATORY MATERIALS

Title of Research: Pamelo (citrus maxima) peel extract as a clearing agent in Tissue Processing
Approximate date of use:

MATERIAL/REAGENT	AMOUNT NEEDED	NOTES	STOCKS
Glass Slides (75 mm by 25mm)	72 pieces		
Compound Microscope			
Thermometer			
Alcohol lamp			
Distillation flask			
Condenser			
Receiving flask			
Holding stand			
Amber sterile bottles	5 pcs		
~~Antimicrobial liquid soap~~	c/o student		
10% hydrogen peroxide	c/o student		
Pasteur pipette ~~s~~	plastic : 10 pcs		
Chloroform	12-15 ml		
Sulfuric acid	12-15 ml		
Tissue Casettes	~~15 pcs~~ 5-6		
Forcep			
Test tubes	12x75 mm		
~~Beaker~~	bottles w/ cover		
Water Bath			
Rotary Microtome			
Xylene	100-200 ml		
Formaldehyde	250-300 ml		
Hematoxylin and Eosin stain	250-300 ml		
Paraffin Wax	~~500~~ grams adjust		
Alcohol Solutions	500 ml	specify concentrations	
Egg Albumin	5mL		

Prepared by:

(Name of Members)

1. Alagna, Jhoana Marie for JMV
2. Almodovar, John Philip
3. Calub, Jhell Ashley
4. Lagarteja, Yvonne G.
5. Maglalang, June Joshua
6. Mercado, Razen B.
7. Sheetak, Heena for JMV

Checked by:

for

BRYLLE KEVIN UGAY, RMT
Research Adviser, CMLS

Confirmed and verified by:

02/03/25

HAIZEL B. BENING, RMT
Laboratory Custodian, CMLS

Noted by:

RYAN JAY G. MOSTOLES, RMT, CT, MASE
Laboratory Courses Coordinator, CMLS

Approved by:

2/4/25

JOSEPHINE C. MILAN, RMT, MSMT
Dean, CMLS

LORMA COLLEGES
Your Pathway to a Brighter Future

LORMA COLLEGES
Carlatan, City of San Fernando
La Union, 2500 Philippines
COLLEGE OF MEDICAL LABORATORY SCIENCE

RESEARCH 2 REQUEST FOR LABORATORY MATERIALS

Title of Research:"Pomelo(*Citrus maxima*) peel extract as a Clearing Agent in Tissue Processing"
Approximate date of use: March 10, 2025

MATERIAL/REAGENT	AMOUNT NEEDED	NOTES	STOCKS
Rotary Evaporator	1		

Prepared by:
(Name of Members)
1. Alagna, Jhoana Marie F.
2. Almodovar, John Philip V.
3. Calub, Jhell Ashley J.
4. Lagarteja, Yvonne G.
5. Maglalang, June Joshua R.
6. Mercado, Razen B.
7. Sheetak, Heena R.

Checked by:
BRYLLE KEVIN UGAY, RMT
Research Adviser, CMLS

3/7/25
JOSEPHINE C. MILAN, RMT, MSMT
Dean, College of Medical Laboratory Science

Confirmed and verified by:
JERALD MACAPAGAL
Laboratory Custodian, COP

Noted by:
CONRADO A. APUSEN III, RPh
Laboratory Courses Coordinator, COP

Approved by:
03.07.2025
ELLEN MAE P. ABIQUI, RPh, MSPharm, CPT
Executive Director, Campus for Health Sciences & Dean, College of Pharmacy

NOTE: March 15, 2025 (1PM onwards)
Breakage fee : 1,000 paid (H)
To pay the additional payment after the procedure.

Appendix M

Plagiarism Certification

POMELO (Citrus maxima) PEEL EXTRACT AS A CLEARING AGENT IN TISSUE PROCESSING

by PRIYA SHEETAK

General metrics

96,045	14,525	1135	58 min 5 sec	1 hr 51 min
characters	words	sentences	reading time	speaking time

Score

This text scores better than 82% of all texts checked by Grammarly

Writing Issues

570	185	385
Issues left	Critical	Advanced

Plagiarism

54 sources

3% of your text matches 54 sources on the web or in archives of academic publications

chap 1

by PRIYA SHEETAK

General metrics

21,696	3,213	210	12 min 51 sec	24 min 42 sec
characters	words	sentences	reading time	speaking time

Score

This text scores better than 84% of all texts checked by Grammarly

Writing Issues

123	34	89
Issues left	Critical	Advanced

Plagiarism

9 sources

1% of your text matches 9 sources on the web or in archives of academic publications

chap 2

by PRIYA SHEETAK

General metrics

25,168	3,795	274	15 min 10 sec	29 min 11 sec
characters	words	sentences	reading time	speaking time

Score

This text scores better than 73% of all texts checked by Grammarly

Writing Issues

235	61	174
Issues left	Critical	Advanced

Plagiarism

9 sources

1% of your text matches 9 sources on the web or in archives of academic publications

chap 3

by PRIYA SHEETAK

General metrics

25,471	3,824	337	15 min 17 sec	29 min 24 sec
characters	words	sentences	reading time	speaking time

Score

This text scores better than 90% of all texts checked by Grammarly

Writing Issues

121	37	84
Issues left	Critical	Advanced

Plagiarism

10
sources

1% of your text matches 10 sources on the web or in archives of academic publications

chap 4

by PRIYA SHEETAK

General metrics

3,597	516	31	2 min 3 sec	3 min 58 sec
characters	words	sentences	reading time	speaking time

Score

This text scores better than 86% of all texts checked by Grammarly

Writing Issues

21	6	15
Issues left	Critical	Advanced

Plagiarism

2 sources

3% of your text matches 2 sources on the web or in archives of academic publications

APPENDIX N

Grading Sheet

INSTRUCTION: Please Grade the Specimen according to the following:

a. Clearing ability through evaluation of the translucency.
b. Evaporation rate in the Paraffin Oven
c. Compatibility with stain
d. Morphology of the chicken liver based on the shrinkage or swelling

Parameter	**Scoring Guidelines**				**Score**
	4	**3**	**2**	**1**	
Clearing ability through evaluation of the translucency.	The tissue is fully translucent with no visible opaque areas, indicating complete clarity. Light passes through the tissue uniformly without obstruction. The structure and finer details are easily discernible.	Tissue exhibits apparent translucency, with small, scattered opaque areas that do not significantly impede light transmission. Most of the tissue maintains clarity, but minor irregularities are present.	Tissue demonstrates moderate translucency, with multiple significant opaque regions that disrupt the passage of light. While some areas allow light through, others remain partially obstructed, limiting the visibility of finer details.	The tissue is predominantly opaque, allowing minimal light transmission. Most areas are non-translucent, obstructing visibility and clarity. Only a tiny fraction of the tissue exhibits slight translucency.	
Evaporation rate in the	High evaporation	Moderate evaporation	Adequate evaporation	Weak or uneven	

Paraffin Oven.	rate with consistent results, demonstrating cleared tissue sample	rate with some variability but still showing cleared tissue samples.	rate with minor inconsistencies in the cleared tissue sample.	evaporation with poorly cleared tissue samples.	
Compatibility with stain	Strong, Uniform staining with clear differentiation of cellular and tissue structures	Moderate Staining with partial uniformity.	Consistent and adequate staining; good differentiation with minor inconsistencies	Weak or Uneven staining; No clear differentiation of structure	
Morphological changes of the chicken liver tissue based on the degree of shrinkage or swelling	Tissue shows no significant shrinkage or swelling; original morphology is perfectly preserved, with structures appearing intact and undistorted.	Tissue shows minimal shrinkage or swelling, with slight changes in size but no significant distortion of morphology or structural integrity.	Tissue exhibits moderate shrinkage or swelling, with noticeable changes in size and some distortion of structures, affecting overall clarity.	Tissue shows severe shrinkage or swelling, leading to significant distortion of structures and unrecognizable morphology.	

Appendix O

Evaluation Results

Clearing ability through evaluation of translucency

	Control 1	Control 2	Control 3	Treatment 1	Treatment 2	Treatment 3
Evaluator 1	2	3	1	T1001=2 T1002=2 T1003=2	T2001=3 T2002=4 T2003=3	T3001=1 T3002=2 T3003=1
Evaluator 2	4	3	2	T1001=2 T1002=3 T1003=3	T2001=4 T2002=4 T2003=4	T3001=2 T3002=3 T3003=1
Evaluator 3	4	3	3	T1001=2 T1002=4 T1003=3	T2001=3 T2002=3 T2003=3	T3001=3 T3002=3 T3003=3
Evaluator 4	3	2	3	T1001=2 T1002=4 T1003=2	T2001=4 T2002=4 T2003=3	T3001=3 T3002=3 T3003=2
Evaluator 5	3	2	1	T1001=3 T1002=3 T1003=3	T2001=3 T2002=3 T2003=3	T3001=2 T3002=2 T3003=2
Total:	16	13	10	T1001= 11 T1002= 16 T1003= 13	T2001= 17 T2002= 18 T2003= 16	T3001= 11 T3002= 13 T3003= 9

Evaporation rate in the Paraffin Oven

	Control 1	Control 2	Control 3	Treatment 1	Treatment 2	Treatment 3
Evaluator 1	2	3	1	T1001=2 T1002=2 T1003=2	T2001=3 T2002=4 T2003=3	T3001=1 T3002=2 T3003=1
Evaluator 2	4	3	2	T1001=3 T1002=2 T1003=2	T2001=3 T2002=3 T2003=3	T3001=1 T3002=1 T3003=2
Evaluator 3	3	3	1	T1001=2 T1002=3 T1003=2	T2001=3 T2002=2 T2003=4	T3001=1 T3002=1 T3003=1
Evaluator 4	3	2	2	T1001=2 T1002=4 T1003=2	T2001=4 T2002=4 T2003=3	T3001=3 T3002=3 T3003=1
Evaluator 5	2	2	2	T1001=2 T1002=3 T1003=2	T2001=2 T2002=2 T2003=2	T3001=2 T3002=1 T3003=1
Total:	14	13	8	T1001= 11 T1002= 14 T1003= 10	T2001= 15 T2002= 15 T2003= 15	T3001= 8 T3002= 8 T3003= 6

Compatibility with stain

	Control 1	Control 2	Control 3	Treatment 1	Treatment 2	Treatment 3
Evaluator 1	2	3	1	T1001=2 T1002=2 T1003=2	T2001=3 T2002=4 T2003=4	T3001=1 T3002=2 T3003=1
Evaluator 2	4	3	3	T1001=2 T1002=2 T1003=2	T2001=4 T2002=3 T2003=2	T3001=2 T3002=4 T3003=1
Evaluator 3	3	3	2	T1001=2 T1002=3 T1003=3	T2001=3 T2002=3 T2003=3	T3001=2 T3002=3 T3003=1
Evaluator 4	2	2	2	T1001=3 T1002=4 T1003=2	T2001=4 T2002=4 T2003=4	T3001=3 T3002=3 T3003=1
Evaluator 5	1	1	1	T1001=2 T1002=2 T1003=3	T2001=2 T2002=3 T2003=3	T3001=2 T3002=2 T3003=2
Total:	12	12	9	T1001= 11 T1002= 13 T1003= 12	T2001= 16 T2002= 17 T2003= 16	T3001= 10 T3002= 14 T3003= 6

Morphological changes of the chicken liver tissue based on degree of shrinkage or swelling

	Control 1	Control 2	Control 3	Treatment 1	Treatment 2	Treatment 3
Evaluator 1	2	3	1	T1001=2 T1002=2 T1003=2	T2001=3 T2002=4 T2003=3	T3001=1 T3002=2 T3003=1
Evaluator 2	4	4	3	T1001=1 T1002=3 T1003=2	T2001=3 T2002=3 T2003=3	T3001=2 T3002=2 T3003=1
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Evaluator 4	3	2	2	T1001=2 T1002=4 T1003=3	T2001=4 T2002=4 T2003=4	T3001=4 T3002=3 T3003=2
Evaluator 5	3	2	1	T1001=3 T1002=3 T1003=2	T2001=3 T2002=3 T2003=3	T3001=3 T3002=2 T3003=2
Total:	16	14	9	T1001= 12 T1002= 16 T1003=13	T2001= 16 T2002= 18 T2003= 16	T3001= 12 T3002= 12 T3003= 7

Appendix P

Computer-Generated Statistical Outputs

Translucency

ANOVA: Single Factor

DESCRIPTION Alpha 0.05

Group	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
CONTROL	15	39	2.6	0.828571	11.6	0.191899	2.215579	2.984421
T1	15	40	2.666667	0.52381	7.333333	0.191899	2.282246	3.051087
T2	15	51	3.4	0.257143	3.6	0.191899	3.015579	3.784421
T3	15	33	2.2	0.6	8.4	0.191899	1.815579	2.584421

ANOVA

Sources	*SS*	*df*	*MS*	*F*	*P value*	*Eta-sq*	*RMSSE*	*Omega Sq*
Between Groups	11.25	3	3.75	6.788793	0.000552	0.266693	0.672745	0.224469
Within Groups	30.93333	56	0.552381					
Total	42.18333	59	0.714972					

TUKEY HSD/KRAMER alpha 0.05

group	*mean*	*n*	*ss*	*df*	*q-crit*
CONTROL	2.6	15	11.6		
T1	2.666667	15	7.333333		
T2	3.4	15	3.6		
T3	2.2	15	8.4		
		60	30.93333	56	3.744714

Q TEST

group 1	*group 2*	*mean*	*std err*	*q-stat*	*lower*	*upper*	*p-value*	*mean-crit*	*Cohen d*
CONTROL	T1	0.066667	0.19189	0.34740	-0.65194	0.78527	0.99473	0.71860	0.08969
CONTROL	T2	0.8	0.19189	4.16885	0.08139	1.51860	0.02343	0.71860	1.07639
CONTROL	T3	0.4	0.19189	2.08442	-0.31861	1.11860	0.45983	0.71860	0.53819
T1	T2	0.73333	0.19189	3.82144	0.01472	1.45194	0.04380	0.71860	0.98669
T1	T3	0.46667	0.19189	2.43182	-0.25194	1.18527	0.32336	0.71860	0.62789
T2	T3	1.2	0.19189	6.25327	0.48139	1.91860	0.00026	0.71860	1.61458

Evaporation

ANOVA: Single Factor

DESCRIPTION Alpha 0.05

Group	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
CONTROL	15	35	2.333333	0.666667	9.333333	0.190238	1.952241	2.714426
T1	15	35	2.333333	0.380952	5.333333	0.190238	1.952241	2.714426
T2	15	45	3	0.571429	8	0.190238	2.618908	3.381092
T3	15	22	1.466667	0.552381	7.733333	0.190238	1.085574	1.847759

ANOVA

Sources	*SS*	*df*	*MS*	*F*	*P value*	*Eta-sq*	*RMSSE*	*Omega Sq*
Between Groups	17.78333	3	5.927778	10.91959	9.48E-06	0.369076	0.853213	0.331542
Within Groups	30.4	56	0.542857					
Total	48.18333	59	0.816667					

TUKEY HSD/KRAMER alpha 0.05

group	*mean*	*n*	*ss*	*df*	*q-crit*
CONTROL	2.333333	15	9.333333		
T1	2.333333	15	5.333333		
T2	3	15	8		
T3	1.466667	15	7.733333		
		60	30.4	56	3.744714

Q TEST

group 1	*group 2*	*mean*	*std err*	*q-stat*	*lower*	*upper*	*p-value*	*mean-crit*	*Cohen d*
CONTROL	T1	0	0.19023	0	-0.71239	0.71238	1	0.71238	0
CONTROL	T2	0.66666	0.19023	3.50438	-0.04572	1.37905	0.074503	0.71238	0.90482
CONTROL	T3	0.86666	0.19023	4.55569	0.15428	1.57905	0.011115	0.71238	1.17627
T1	T2	0.66666	0.19023	3.50438	-0.04572	1.37905	0.074503	0.71238	0.90482
T1	T3	0.86666	0.19023	4.55569	0.15428	1.57905	0.011115	0.71238	1.17627
T2	T3	1.53333	0.19023	8.06008	0.82094	2.24572	2.75E-06	0.71238	2.08110

Shrinkage or Swelling

ANOVA: Single Factor

DESCRIPTION Alpha 0.05

Group	*Count*	*Sum*	*Mean*	*Variance*	*SS*	*Std Err*	*Lower*	*Upper*
CONTROL	15	39	2.6	0.971429	13.6	0.220389	2.158507	3.041493
T1	15	41	2.733333	0.923810	12.93333	0.220389	2.291840	3.174827
T2	15	50	3.333333	0.238095	3.333333	0.220389	2.891840	3.774827
T3	15	31	2.066667	0.780952	10.93333	0.220389	1.625173	2.508160

ANOVA

Sources	*SS*	*df*	*MS*	*F*	*P value*	*Eta-sq*	*RMSSE*	*Omega Sq*
Between Groups	12.183333	3	4.061111	5.574034	0.002034	0.229947	0.609547	0.186134
Within Groups	40.8	56	0.728571					
Total	52.983333	59	0.898023					

TUKEY HSD/KRAMER alpha 0.05

group	*mean*	*n*	*ss*	*df*	*q-crit*
CONTROL	2.6	15	13.6		
T1	2.733333	15	12.93333		
T2	3.333333	15	3.333333		
T3	2.066667	15	10.93333		
		60	40.8	56	3.744714

Q TEST

group 1	*group 2*	*mean*	*std err*	*q-stat*	*lower*	*upper*	*p-value*	*mean-crit*	*Cohen d*
CONTROL	T1	0.13333	0.22089	0.6049	-0.6919	0.95828	0.97376	0.82529	0.15620
CONTROL	T2	0.73333	0.22089	3.3274	-0.0919	1.55828	0.09842	0.82529	0.85914
CONTROL	T3	0.53333	0.22089	2.4199	-0.2919	1.35828	0.32762	0.82529	0.62483
T1	T2	0.6	0.22089	2.7224	-0.2252	1.42595	0.22939	0.82529	0.70293
T1	T3	0.66667	0.22089	3.0249	-0.1586	1.49261	0.15349	0.82529	0.78103
T2	T3	1.26667	0.22089	5.7474	0.44137	2.09196	0.00085	0.82529	1.48397

Appendix P

Experimentation and Data Gathering Protocol

POMELO (*Citrus maxima*) PEEL EXTRACT AS A CLEARING AGENT IN TISSUE PROCESSING

Materials

- Compound Microscopy
- Amber sterile bottle
- Antimicrobial Liquid Soap
- 10% Buffered formalin
- 10% Hydrogen Peroxide
- Pasteur Pipette
- Rotary Microtome
- Tissue Cassettes
- Hematoxylin and Eosin Stain
- Paraffin Wax
- Rotary Evaporator
- Forceps
- Chopping Board
- Knife
- Test Tubes
- Egg Albumin
- Paraffin Oven
- Xylene
- Ascending Alcohol Solutions
- Electric Scale
- Distilled Water
- Microscopic Slides (75 mm by 25 mm)
- 500 mL Jar

D-Limonene Extraction

Materials:

- Distilled Water
- Pomelo peel
- Screw top-lid container
- Rotary Evaporator
- Gauze
- Basin
- Filter Paper

1. Separate the pomelo peel from the fruit and pulp. The pomelo must be washed with antimicrobial liquid soap.
2. Cut the pomelo peels into tiny slices and wash with distilled water again and then macerate with distilled water for 4 days.
3. Filter the macerated extract using gauze and perform the rotary evaporation to obtain the crude extract.
4. Filter the crude extract using filter paper and let the extract stand for 2 days to let the limonene float.

Miscibility Testing

Materials:

- Test Tube
- 100% Ethanol
- Limonene
- Pasteur Pipette

1. Three different ratios of D-limonene to ethanol were tested to determine the miscibility changes with different amounts of ethanol.
2. 1 mL of D-Limonene was mixed with 1 mL of ethanol (1:1) in test tube 1.
3. In test tube 2, 2 mL of D-limonene was mixed with 1 mL of ethanol (2:1).
4. And in test tube 3, 1 mL of D-limonene was mixed with 2 mL of ethanol (1:2).

Salkowski Testing

Materials:

- Test Tube
- Chloroform
- Sulfuric acid
- Limonene

1. The extracted D-limonene from pomelo peel were subjected to Salkowski testing to confirm its presence.
2. A 5 mL of the extract was mixed in a 2 mL chloroform and 3 mL concentrated Sulfuric acid to form a layer.
3. A pale yellow to reddish brown coloration will show a positive result for the presence of D-limonene (Karthika, et. al, 2022) Salkowski testing was utilized in the confirmation of presence of D-limonene.

Tissue Processing

Materials:

- Tissue Cassettes
- Paraffin Oven
- Paraffin Wax
- Xylene
- D-Limonene
- 70% Ethanol, 80% Ethanol, 95% Ethanol and 100% Ethanol
- 500 mL Jars
- 10% Buffered Formalin
- Paper Boats

1. Pre-Fixed the Chicken Liver for transportation and grossed the liver for preparation of fixation.
2. Fixed the grossed chicken liver using 10% buffered formalin for 24 hours.
3. Perform the dehydration in ascending alcohol: immerse the tissue liver in 70% ethanol for 1 hour, then transfer in 80% ethanol for another hour, then transfer in 95% ethanol for another hour and transfer in 100% ethanol for another hour.
4. Clear the tissue sample using xylene for control and use the limonene for the different treatments.
5. Infiltrate the cleared tissue in melted paraffin wax for 1 hour and agitate every 15 minutes.
6. Embed the infiltrated tissue samples and let it cool at room temperature
7. Section the embedded tissue sample in ribbons
8. Mount the ribbons in the slide and let the slide dry.
9. Stain the mounted tissue samples.
10. Apply the cover slip using eukitt mounting media
11. Evaluate the tissue samples using compound microscope

Appendix R

Time Table

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

GENERAL RESEARCH TIMETABLE CHART

Research Title: Pomelo (*Citrus maxima*) Peel Extract as a Clearing Agent in Tissue Processing

Researchers: Jhoanna Marie F. Alagna, John Philip V. Almodovar, Jhell Ashley J. Calub, Yvonne G. Lagarteja, June Joshua R. Maglalang, Razen B. Mercado, and Heena R. Sheetak

Research Advisers: Brylle Kevin Ugay, RMT

Timetable	Research Task	Methodology/Strategy/ Technique	Status
09/05/24	Formulation of Research Title	Face to Face meeting, Messenger Chat, Encoding through laptop	Completed
09/07/24	Presentation of Research Title	Face to Face meeting	Completed
10/11/24	Formulation of Research Process	Messenger chat, Face to face Meeting, Encoding through laptop	Completed
10/16/24	Accomplishment of Chapter 1	Google Meeting	Completed
10/22/24	Revision of the Research Paradigm	Messenger chat Google Document Encoding through laptop	Completed
10/22/24	Revision of Research Problem	Messenger chat Google Document Encoding through laptop	Completed
10/22/24	Revision of Hypotheses	Messenger chat Google Document Encoding through laptop	Completed
10/26/24	Revision of Research Theoretical/Conceptual	Messenger chat Google Document	Completed

	Framework	Encoding through laptop	
10/31/24	Revision on Background of the Study	Messenger chat Google Document Encoding through laptop	Completed
10/31/24	Revision of Research Theoretical/Conceptual Framework	Messenger chat Google Document Encoding through laptop	Completed
11/14/24	Initial Review for Chapter 1	Google Document Encoding through laptop	Completed
11/16/24	Revision for Chapter 1	Messenger chat Encoding through laptop	Completed
11/16/24	Accomplishment of Chapter II	Google Document Messenger Chat encoding through laptop	Completed
11/17/24	Revision for Chapter 1 Accomplishment of Chapter 2	Google Document Encoding through laptop	Completed
11/20/24	Accomplishment of Chapter 1 Start of Chapter 2	Google Document Encoding through laptop	Completed
11/21-25/24	Accomplishment of Chapter 2	Google Document Encoding through laptop	Completed
11/26/24	Feedback for Chapter 2	Google Document Encoding through laptop	Completed
11/28-30/24	Revision of Chapter Editing of Chapter 1	Google Document Encoding through laptop	Completed
12/2/24	Feedback for Chapter 1	Google Document	Completed

	and 2	Encoding through laptop	
12/3/24	Revision of Chapter 1 and 2	Google Document Encoding through laptop	Completed
12/4/24	Revision of Chapter 1 and 2	Google Document Encoding through laptop	Completed
12/5/24	Revision of Chapter 1 and 2	Google Document Encoding through laptop	Completed
12/6/24	Revision of Chapter 1 and 2	Google document Encoding through laptop	Completed
12/7/24	Revision of Chapter 1 and 2	Google document Encoding through laptop	Completed
12/8/24	Feedback for Chapter 2 Revision of Chapter 2	Google Document Encoding through laptop	Completed
12/9/24	Finalization of Chapter 1 and 2	Google Document Encoding through laptop	Completed
12/10/24	Finalization of Chapter 1 and 2	Google Document Encoding through laptop	Completed
12/11/24	Finalization of Chapter 2 and 2	Google Document Encoding through laptop	Completed
12/12/24	Finalization of Chapter 1 and 2 Mock Defense	Google Document Encoding through laptop Google Meeting	Completed
12/14/24	Proposal Defense and Defense Proper	Face to Face	Completed

12/15/24	Revision of the paper based on the proposal defense	Google Document Encoding through laptop	Completed
12/16-20/24	Revision of the paper based on the proposal defense	Google Document Encoding through laptop	Completed
12/21/24	Submission of Revised Research Paper	Passed in the Faculty	Completed
01/22/2025	Passing of the requirements in the REC for approval	Google Document	Completed
01/28/2025	Requesting of the laboratory materials	Google Documents Passed to Ms. Bening	Completed
01/31/2025	Approval of the Research Study	Email	Completed
02/07/2025	Preparation of Plant identification letter	Google Documents Signatories of the research advisers and the dean of CMLS	Completed
02/27/2025	Preparation of Letter to the College of Pharmacy for Laboratory materials and for Mr. Jerald's Guidance	Google Documents Signatories of the research advisers and the dean of CMLS Passed to Ma'am Abiqui	Completed
02/27/2025	Consultation with Mr. Baladad with the research progress	Face to Face	Completed
02/27/2025	Passing of letter in DMMMSU NLUC for the plant identification	Passed the letter to the chancellor of DMMMSU	Completed
03/03/2025	Process the deposit for breakage amounting 1000 pesos	Pay the breakage deposit and passed to Ma'am Abiqui	Completed
03/04/2025	Consultation of the extraction process in the	Face to Face consultation with	Completed

	College of Pharmacy	Mr. Jerald Macapagal	
03/04/2025	Approval of the request material in the College of Pharmacy	Signatories of the custodian, laboratory coordinator and the dean of COP	Completed
03/05/2025	Certify the Plant Identification Letter	Face to face Get the certificate from Ms. Olbinado from DMMMSU NLUC	Completed
03/07/2025	Canvassing of jars needed for the study	Face to face	Completed
03/10/2025	Peeling of the Pomelo	Peeled through knife	Completed
03/10/2025	Preparation of the Maceration process	Pomelo peels that is washed with distilled water is put in a clear container	Completed
03/11/2025	Maceration process proper	The macerated peels is sent to the laboratory under the supervision of Mr. Jerald Macapagal	Completed
03/13/2025	Consultation with Mr. Jose Sumaya regarding tissue processing	Face to Face	Completed
03/15/2025	Distillation proper and extraction	Face to face Skylab	Completed
03/15/2025	Letting the crude extract rest for the D-Limonene to float	Face to Face	Completed
03/15/2025	Canvassing ng absolute ethanol alcohol	Online (Shopee)	Completed
03/18/2025	Transfer d limonene to amber bottle	Face to Face	Completed
03/18/2025	Salkowski testing and Miscibility testing	LB 101	Completed

03/24/2025	Pre-fixation Grossing Fixation	LB 101	Completed
03/25/2025	Dehydration Clearing Infiltration Embedding	LB 101	Completed
03/27/2025	Sectioning Mounting Staining	LB 101	Completed
03/28/2025 to 04/10/2025	Evaluation of the Slides	LB 101 Microscopy	Completed
04/11/2025	Tallying of the Scores	Google Sheets	Completed
04/13/2025	Statistical Data	Mr. Jose Enrico Sumaya	Completed
04/15/2025 to 04/22/2025	Writing of Chapter 3 and 4	Face to Face and Online Google Docs	Completed
04/26/2025	Final Defense	Oral Face To Face	Completed
04/27/2025 to 04/29/2025	Revision of the Papers	Face to Face Google Docs	Completed
04/30/2025	Passing of the revised paper	Face to face	Completed

Appendix S

Pictures of Experimental Set-Ups

Collection of Pomelo

Sterilization of the Sink

Sterilization of the materials using antimicrobial liquid soap

Sterilization of the materials using 10% hydrogen peroxide

Cleaning of the Pomelo

Removing of the Pomelo peel from the Fruit

Slicing the pomelo peel

Measuring of distilled water used as a solvent for maceration

Maceration Process

Filtration of the Extract using gauze

Rotary Evaporation Process

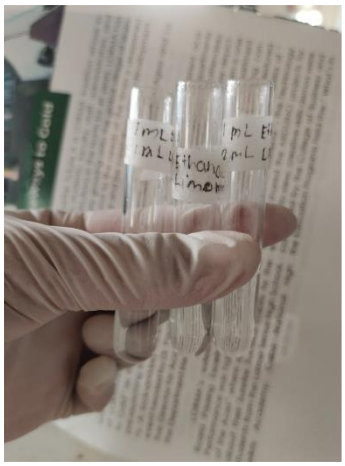
Filtration of the crude extract with filter paper

Letting the extract stand for the limonene to float

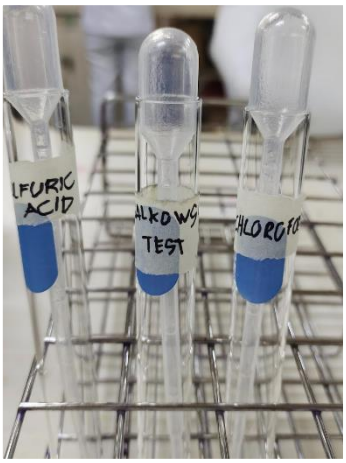
Equipment Sterilization using 10% hydrogen peroxide

Collected D-Limonene





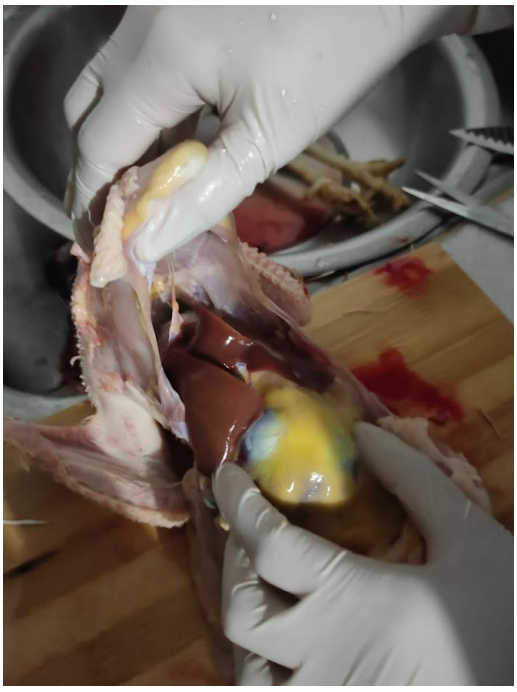
Miscibility Testing




Salkowski Testing

Slaughtering of the Live Chicken

Slaughtered Chicken

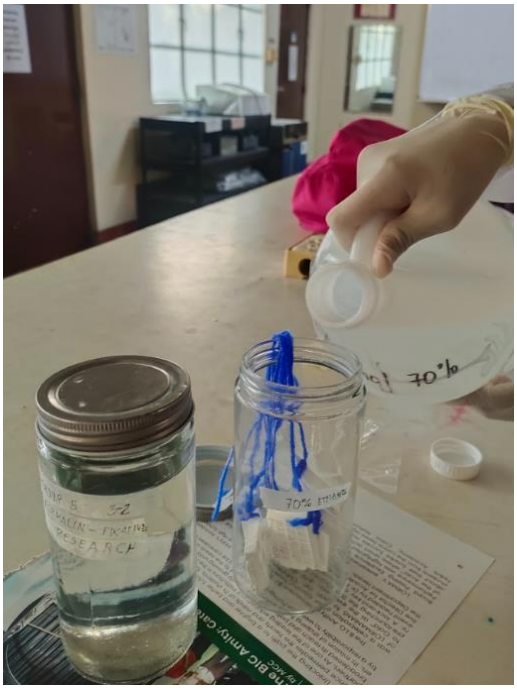


Collection of the Chicken Liver

Pre-Fixation of the Chicken Liver for Transportation

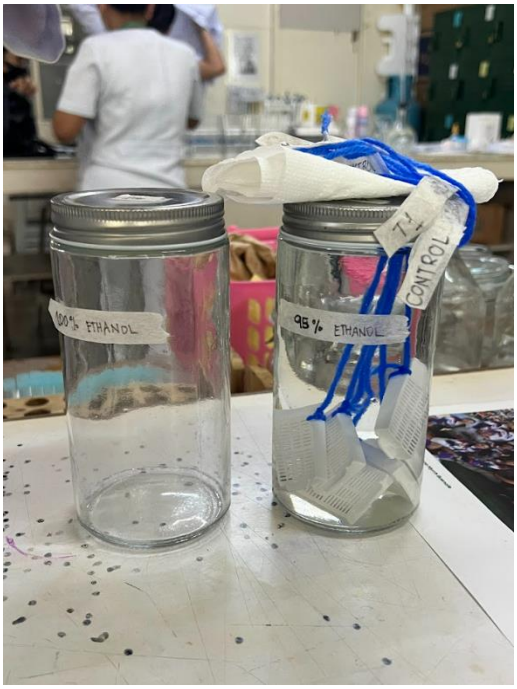
Grossing of the Chicken Liver

Fixation of the Chicken Liver



70% Dehydration

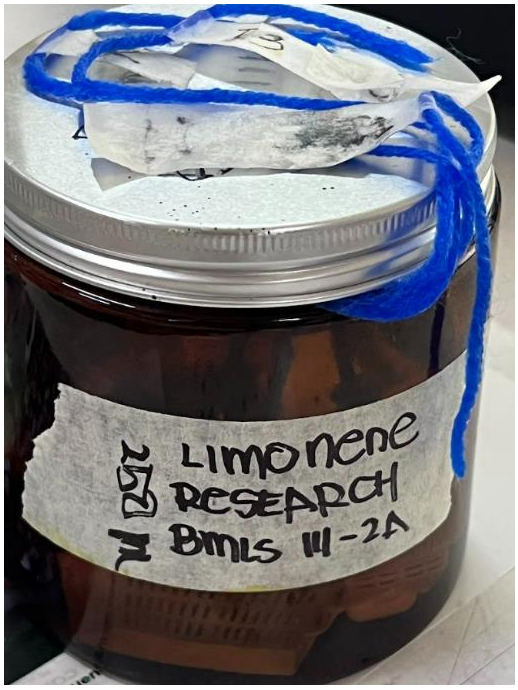
80% Dehydration



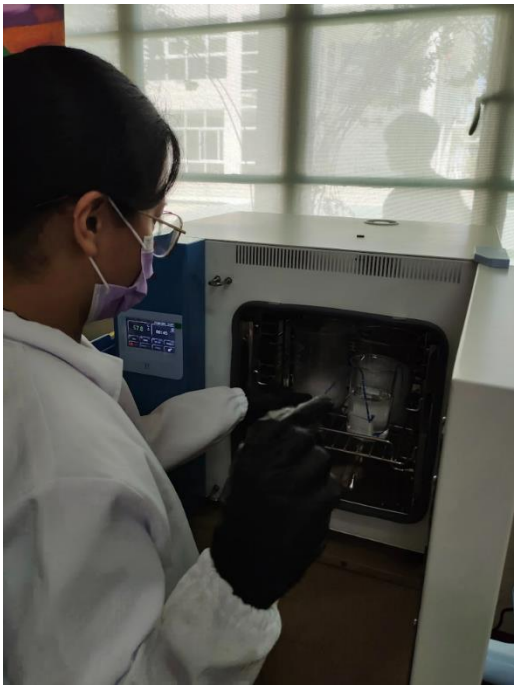
95% Dehydration

100% Dehydration

Clearing Using Xylene



Clearing using the D-limonene



Paraffin Infiltration

Embedding

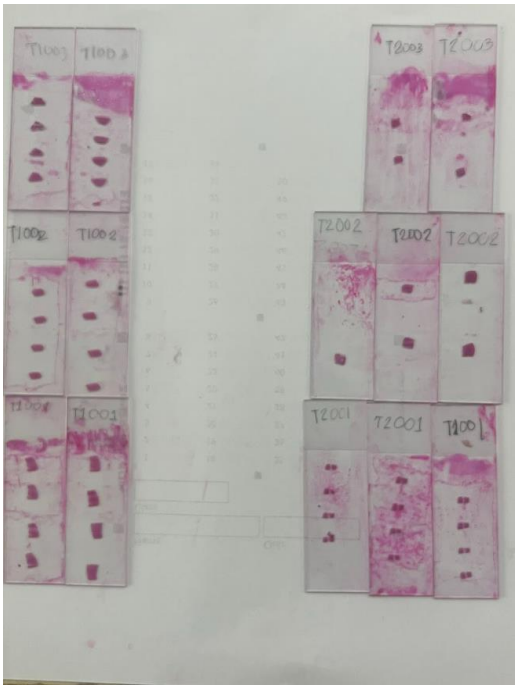
Embedded Tissue

Sectioning

Mounting

H&E staining

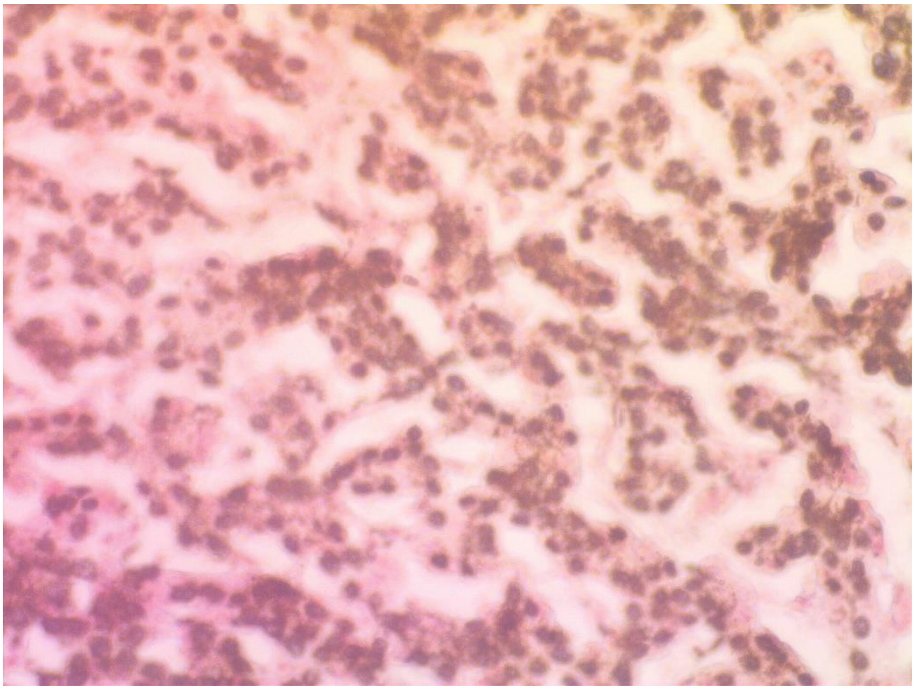
Putting cover slips



Prepared Slides

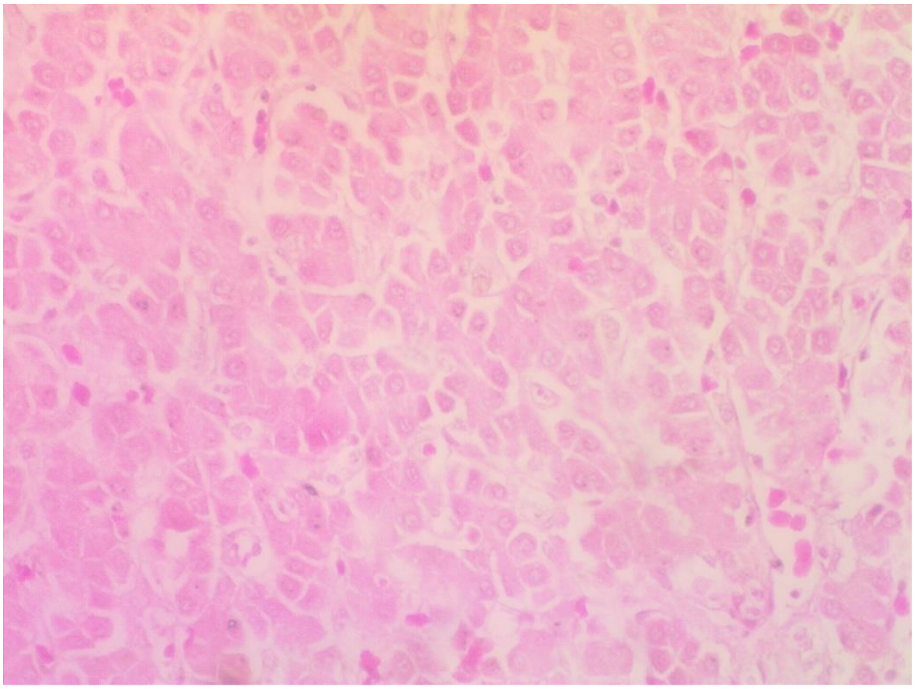
Appendix T

Tissue Micrographs



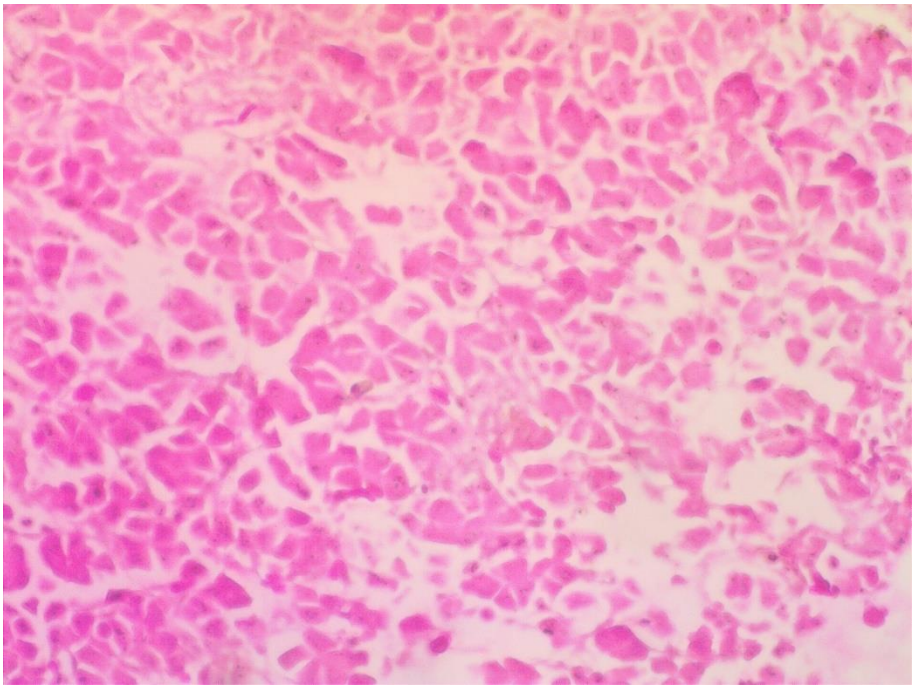
C1 (Control 1)

Nuclear and Cytoplasmic differentiation can be observed. Was able to take up the basic dyes



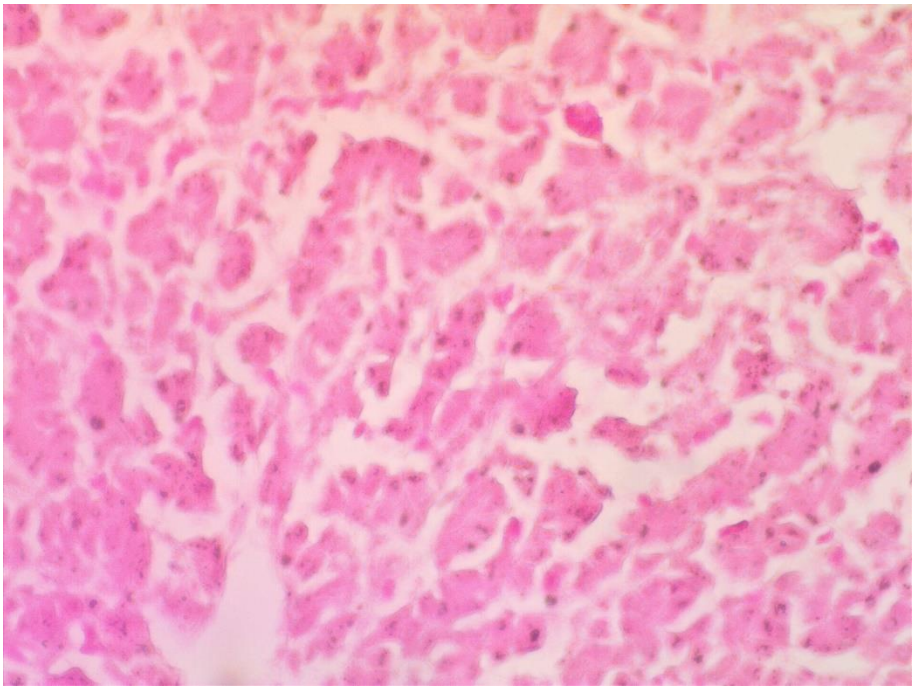
T1001

The nuclear and cytoplasmic differentiation is poor. Did not uptake the basic dye



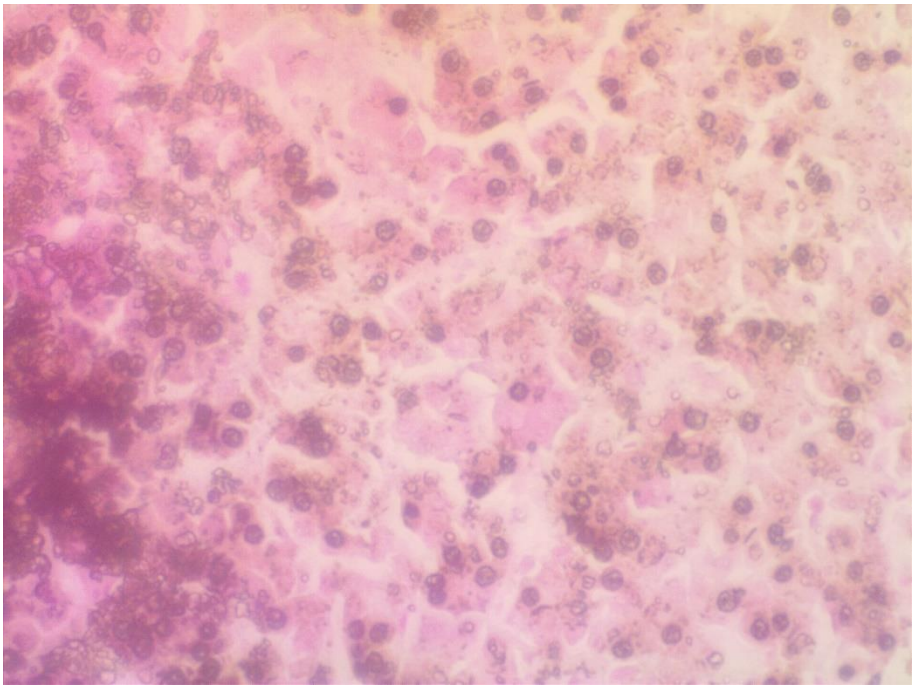
T1002

The nuclear and cytoplasmic differentiation is poor. The color is too pink



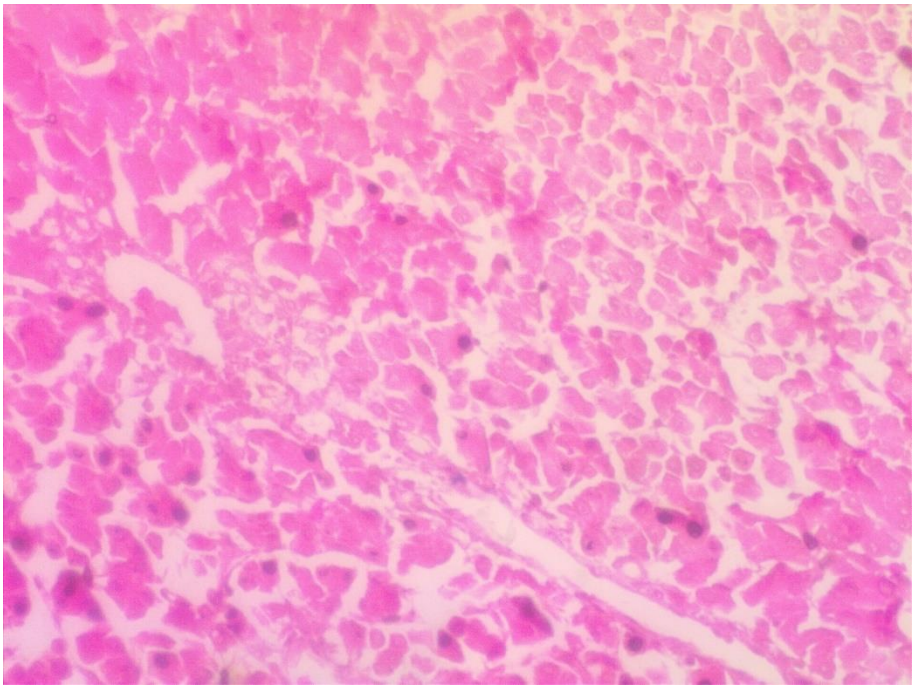
T1003

The tissue shrinks and a poor nuclear and cytoplasmic differentiation is observed.



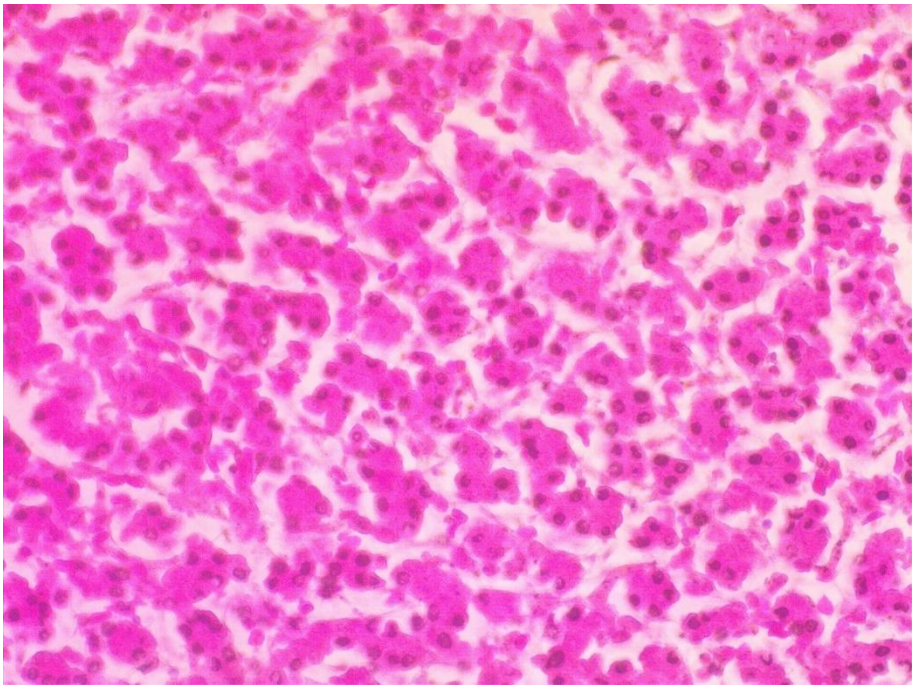
C2 (Control 2)

Nuclear and Cytoplasmic differentiation can be observed. Was able to take up the basic dyes



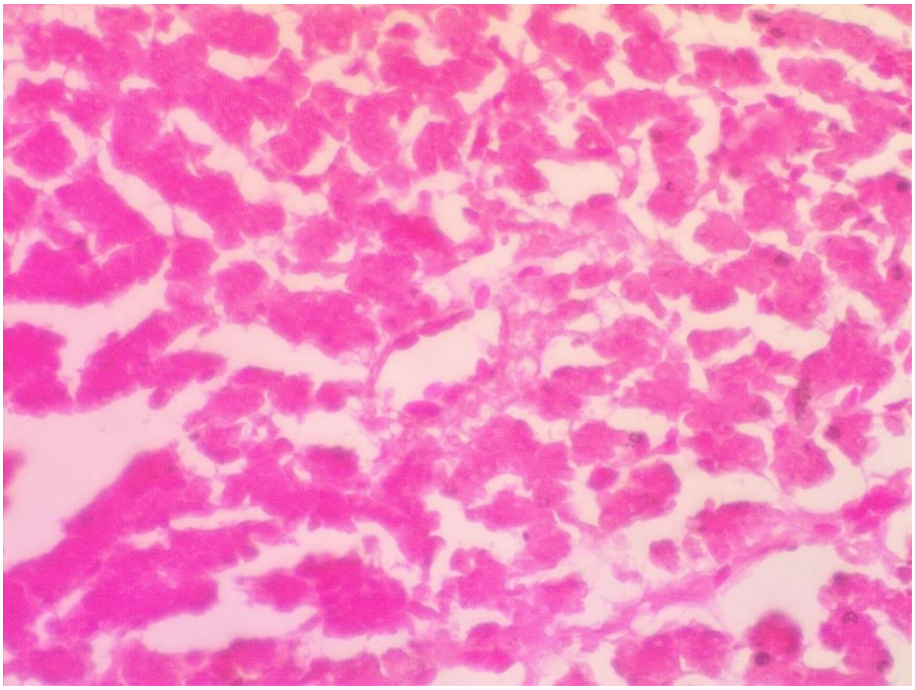
T2001

Some of the nuclei are stained. The cytoplasm was stained too much pink.



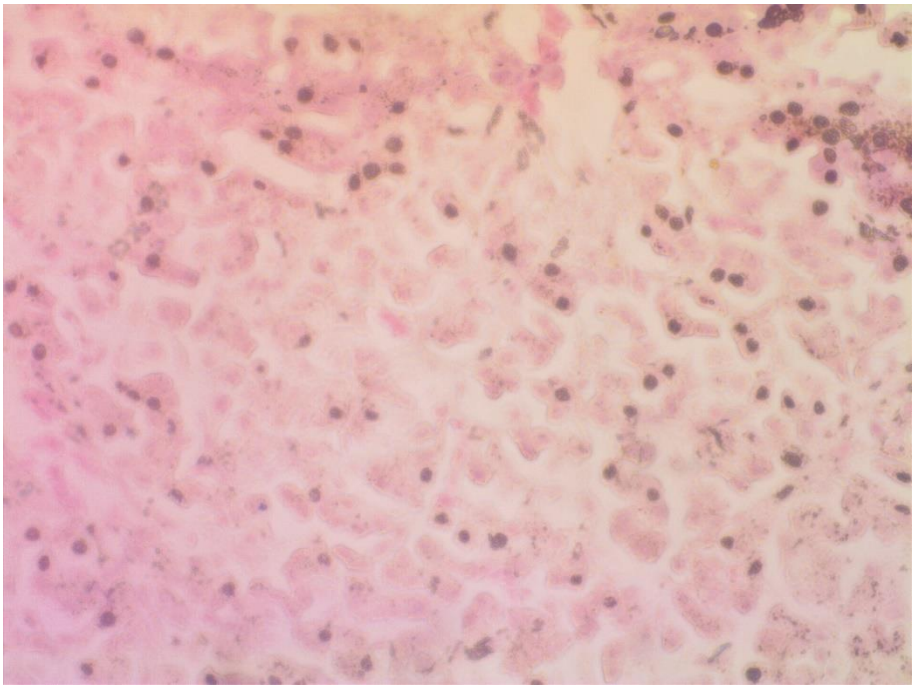
T2002

Most of the nuclei are stained but it did not show a clear cytoplasmic to nuclear differentiation. The color of the specimen is too pink.

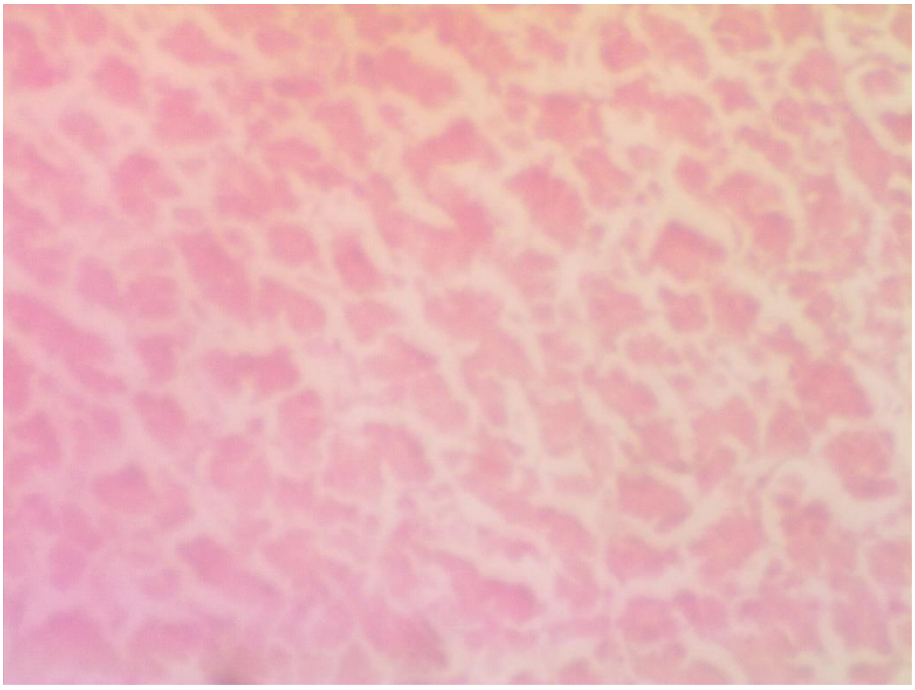


T2003

The nuclei are not stained leading to poor differentiation of the nuclear to cytoplasmic ratio. The color of the specimen is mostly pink.



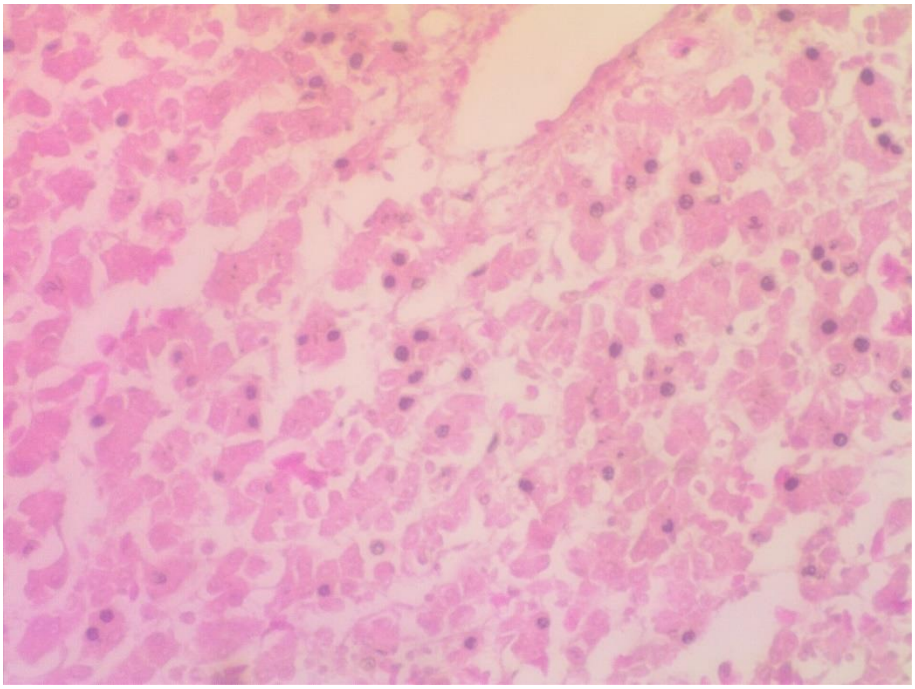
C3 (Control 3)



T3001

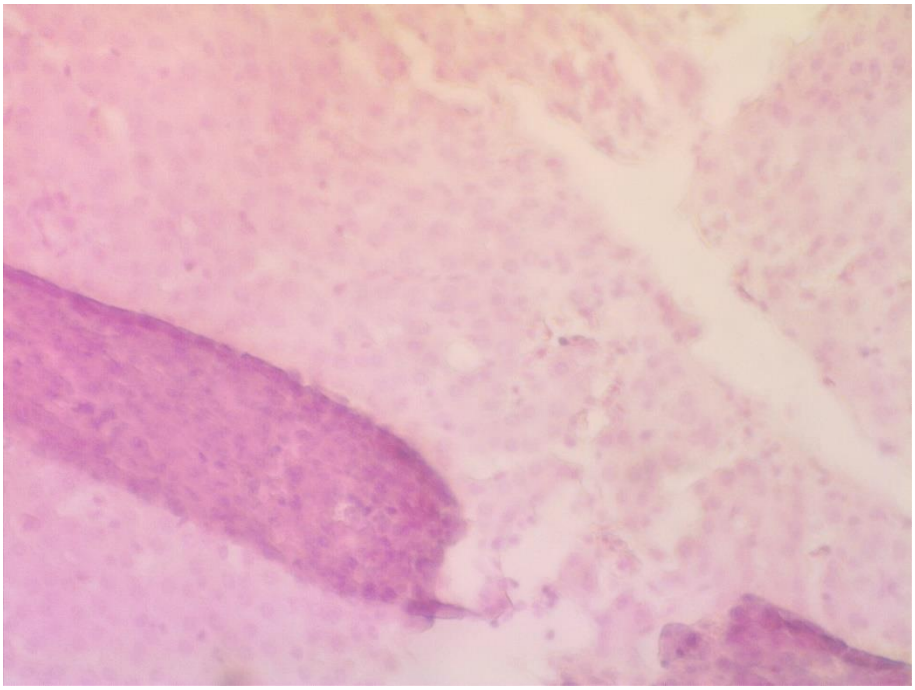
The nuclear and cytoplasmic differentiation and the nuclei are very poor. It did not uptake the basic dye

ss



T3002

The nuclear and cytoplasmic differentiation and the nuclei are very poor. The color of the specimen is pale compared to Treatment 1 and 2



T3003

The nuclear and cytoplasmic differentiation and the nuclei are very poor. It turns out to be pale pink

BIODATA

The researchers are third-year students pursuing a Bachelor of Medical Laboratory Science at Lorma Colleges. They are dedicated to conducting sustainable research aimed at enhancing laboratory practices. Their work aspires to guide future studies and contribute to the continuous improvement of education and research within the Medical Laboratory Science field.