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Potential Wound Healing Activity of Methanolic Root Extract of *Stachytarpheta jamaicensis* Ointment in Male Swiss Albino Mice

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APPROVAL SHEET

This thesis entitled:

"Potential Wound Healing Activity of Methanolic Root Extract of *Stachytarpheta jamaicensis* Ointment in Male Swiss Albino Mice "

Prepared and submitted by Princess Janina D. Anggulo, Railyn L. Daud, Wagne Dale J. Garcesa and Juhaina T. Muripaga has been examined, and is recommended for approval and acceptance as partial fulfillment of the requirements for the degree Bachelor of Science in Pharmacy.

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CERTIFICATE OF ORIGINALITY

We hereby declare that this submission is our own work and that, to the best of our knowledge and belief, it contains no materials previously published or written by another person nor material to which to a substantial extent has been accepted for award of any other degree or diploma of a university or other institute of higher learning, except where due acknowledgement is made in the text.

We also declare that the intellectual content of this thesis is the product of our work, even though we have received assistance from others on style, presentation language.

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It was in this period that we felt God/Allah's guiding hand at work. His blessings illuminated our path and exposed the complex web of truth, hidden behind the complexity of our investigation. We recognize that God/Allah, the source of all wisdom, has blessed us with what we know. Therefore, we kneel down, express thanks for these blessings, and promise to use this knowledge solely for the advancement of knowledge and humanity.

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ABSTRACT

This study were investigated the effectiveness of *S. jamaicensis* root for wound healing. Despite the plant's historical use in traditional medicine practice, scientific evidence were limited, particularly concerning the root. To address this, the researchers evaluated the methanolic root extract's efficacy in promoting wound closure and tissue regeneration in an animal model. The extract, prepared by macerating dried root using methanol, were applied topically to excised wounds in male albino mice. Results were compared to a positive control, Povidone Iodine, and a negative control, Base formulation. Among all comparisons, only the difference between the Negative Control and the 10% concentration was found to be statistically significant with a *p*-value of 0.0436, which is less than the 0.05 significance level. The result of the phytochemical of flavanoids, terpenoids, phenols, tannins was positive and the 10% concentration of every group was received the significant difference in the p value of 0.05 with the p value 0.0436

Keywords: *Root Crude Extract of S. jamaicensis, Povidone Iodine, Excision Model.*

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CHAPTER ONE
THE PROBLEM AND ITS BACKGROUND

This chapter presented an overview of the potential wound-healing activity of the ointment made from the methanolic extract of *Stachytarpheta jamaicensis* (Gervao) root on the excisional wounds of Swiss albino mice. It provided the background of the study, its conceptual framework, the research paradigm, research objectives, proposed hypotheses, the significance of the study, its scope and delimitations, and definitions of terms.

➢ *Introduction*

The skin was a fundamental part of the human body, the largest organ, and the first defense barrier against the environment. When the skin was wounded, the barrier was broken. It could have been a simple surface injury or quite complicated, especially when there were other factors like chronic diseases. Hence, wound healing was a broad topic in healthcare. The biology of wound healing was paced through four phases: hemostasis, inflammation, proliferation, and remodeling (Monica et al. 2022).

A wound was a break in the normal barrier and functions of the skin, usually from some physical trauma, like cuts and scrapes, although it could have been from a chemical injury like a burn from a strong acid or alkali. When the skin structure was compromised or wounded, the normal body response was to begin the healing process immediately. This restored the organism's homeostasis. Much research had focused on finding natural products to aid in wound healing, tapping into traditional practices, and looking for affordable options for this common problem. Studies often showed that healing happened because of the inherent properties of these products, such as the protective work of antioxidants or anti-inflammatory and antimicrobial properties (Criollo-Mendoza et al. 2023).

Wounds could have been classified as acute, occurring with sudden trauma or even planned surgery, and healing rapidly. Other wounds were chronic, healing slowly due to poor tissue health in conditions like diabetes, infection, poor blood supply, or malnutrition that disrupted the healing process. The treatment of wounds was of great importance for human health since the lack of healing and the prolongation of treatment time caused increased suffering and economic costs (Criollo-Mendoza et al. 2023).

Wounds were considered chronic if they did not start to heal within 4 to 12 weeks despite treatment. Usually, treatment took a long time and could have been painful. It was important to treat the underlying condition that contributed to the development of the wound (Cologne and Germany, 2022). The difficulties presented by acute and chronic wounds, which resulted from a variety of sources such as prolonged inflammation, probably continued to be important in both public health and medical practice in the future (Alherz et al. 2022).

From a historical perspective, other species of Verbenaceae, the family to which *S. jamaicensis* belonged, had been used by folk medicinal practitioners (Hamilton et al., 2023). *S. jamaicensis* contained phenolic and flavonoid compounds with antibacterial, antiinflammatory, and antioxidant properties that reduced infections and oxidative stress, promoting faster tissue regeneration and scab formation (Caluya et al., 2017). Tannins further aided healing through their antibacterial, astringent, and antioxidant effects, protecting tissues from oxidative damage (Akanji & Sonibare, 2018). Terpenoids, notably limonin, inhibited pro-inflammatory cytokines, fostering an optimal environment for early tissue repair (Usman et al. 2024).

➢ *Background of the Study*

This research recognized the pressing need for effective strategies in wound healing that were economical and accessible to the average person, as many commercial wound preparations were expensive. In that light, since the medicinal value of *S. jamaicensis* leaves had received much attention in the past, this study evaluated the potential wound-healing activity of the root crude extract of *S. jamaicensis*. This was a comparative study to povidone iodine ointment, as this was one of the most commonly used topical antiseptics.

Flavonoids possessed wound-healing properties due to their well-acclaimed antiinflammatory, angiogenesis, re-epithelialization, and antioxidant effects. They had been shown to act on the wound-healing process via the expression of biomarkers respective to the pathways (Zulkefli et al. 2023).

Polyphenol was a versatile green phytochemical vital in several biomedical applications with fascinating inherent biocompatible, bioadhesive, antioxidant, and antibacterial properties. Effective wound dressings necessitated structures and therapeutic substances with multifunctional properties to achieve rapid wound healing, and polyphenolloaded nanofibers emerged as valuable structures (Ejiohuo et al. 2024).

Tannin also led to improved wound healing and reduced scar tissue formation by inhibiting the formation and removal of reactive oxygen substances. Additional advantages of tannins included relief of pain, limitation of secondary infection, prevention of plasma loss, and promotion of prolific epithelialization (Chokotho et al.2005).

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Terpenoids were also reported to promote wound healing primarily due to their astringent and antimicrobial properties, which could have been responsible for wound contraction and an improved rate of epithelialization (Asumang et al. 2021).

Ointments provided moisture to wounds, which was crucial for faster healing, and created a protective barrier against bacteria. Ointments displayed a significant improvement in wound healing with an earlier onset of re-epithelialization, faster wound closure, and a better outcome (Khulman et al.2019).

White petroleum jelly was generally recommended for patients receiving moist wound healing because of its safety and moisturizing qualities (Yamamoto et al. 2022).

➢ *Conceptual Framework*

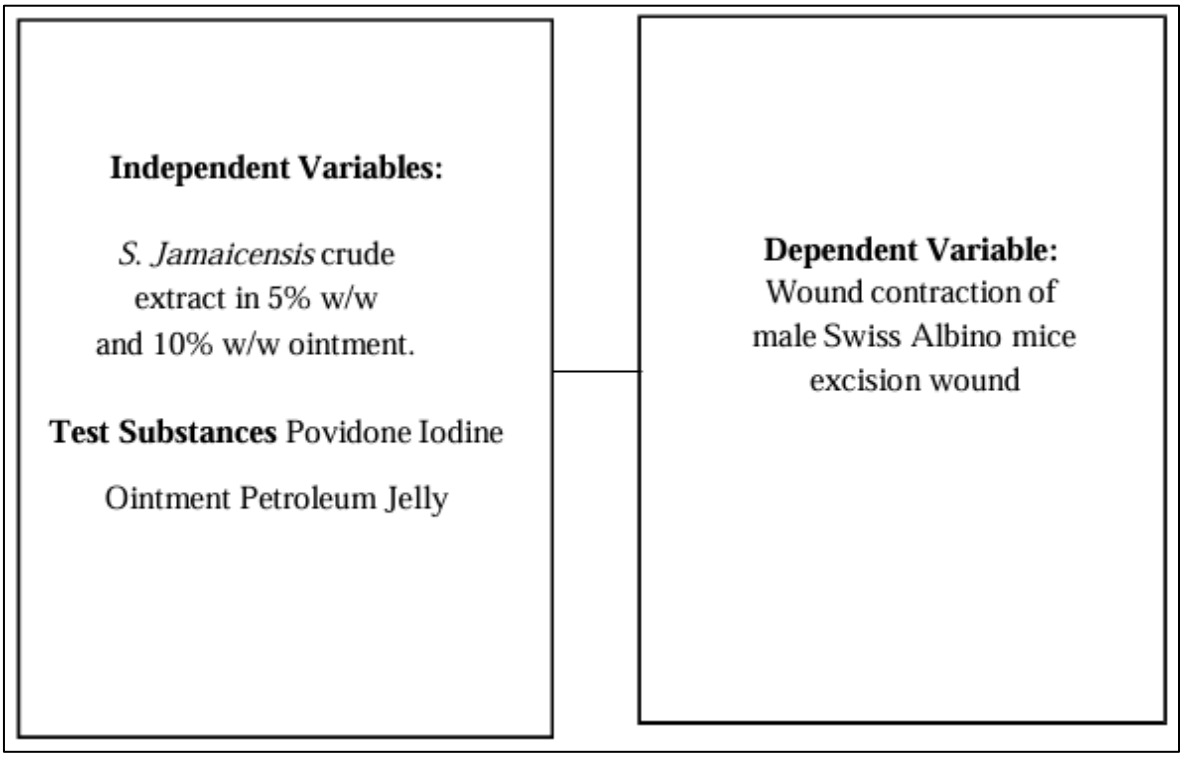

Fig 1 Flow of Research

This study was designed to compare the effects of *S. jamaicensis* at concentrations of 5% w/w and 10% w/w. These percentages were based on previous studies with *S. jamaicensis* leaves and seed pods of other similar plants. The wound healing activity of these two concentrations was compared with the positive control of Povidone Iodine ointment. The healing process was measured on selected days 0, 3, 7, and 14 (measurements of wound contraction). The independent variables, *S. jamaicensis* ointment and the test substances, and the dependent variable, wound contraction, were correlated graphically and analyzed statistically for significance of differences. The results were expected to highlight the effectiveness of using *S. jamaicensis* as a natural product for wound healing.

➢ *Research Objectives*

The design of the research sought to minimize other variables by using all ointment formulations of the controls, by using the punch biopsy to standardize the size of wounds at 8mm, and by measuring the amount of ointment applied 0.1ml each time using a tuberculin syringe in application. In this way, the researchers were able to focus on the effect of varying concentrations of *S. jamaicensis* on wound healing.

In general, this research provided a brief view of the potential of *S. jamaicensis* ointment as a natural and readily available topical formulation that effectively promoted wound healing.

The overall objective of this study was to verify that the crude extract of *S. jamaicensis* aided in the wound-healing process in excisional wounds of mice. To reach this objective, this study included the following objectives:

- To identify and authenticate the *S. jamaicensis* plant specimen.
- To perform a confirmatory qualitative test for the presence of phytochemicals in the extract of *S. jamaicensis*.

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- To formulate *S. jamaicensis* ointment from the extract in two concentrations and a positive control.
- To formulate base ointment as a negative control.
- To perform dermal toxicity testing of the ointment produced.
- To determine the wound-healing activity of the *S. jamaicensis* ointment.
- To statistically analyze the difference in wound healing of the two *S. jamaicensis* formulations compared with the control groups.

➢ *Research Hypothesis*

- H1: The ointment of *S. jamaicensis* is effective as a wound healing agent of excisional wounds in male Swiss albino mice.
- H0: The ointment of *S. jamaicensis* did not demonstrate an accelerated healing effect on wounds in male Swiss albino mice.

➢ *Significance of the Study*

This study was valuable for pharmacy students, the community, and future researchers.

- Pharmacy students: The conclusions of this research were important educational resources, broadening their understanding of alternative treatments for wounds. This deeper knowledge not only advanced their studies but also prepared them to effectively integrate traditional remedies with modern pharmaceutical approaches in their future pharmacy practice.
- Community: This study particularly benefited indigenous communities, who often relied on traditional medicine, by validating *S. jamaicensis* as effective in accelerating wound healing.

By scientifically proving the plant's effectiveness, the study offered a culturally relevant and accessible solution that could improve health outcomes and provide a sustainable method for managing wounds.

- Future researchers: This study inspired further exploration into *S. jamaicensis* and its potential in wound care and other health applications. The insights gained from this research contributed to a better understanding of the plant's benefits and encouraged continued investigation into its potential applications for wound care and other health-related areas.

➢ *Scope and Delimitation*

This study aimed to evaluate the potential healing activity of *S. jamaicensis* root extract ointment on excision wounds in specifically male Swiss albino mice. Males were chosen to avoid other variables related to differences in female wound healing. The study specifically used an excision wound model to assess the impact of the root extract on wound closure size. The observation period for wound healing was limited to 14 days. Though other research efforts had continued their data collection for 21 days, this research followed the example of a study by Tessem and Mola (2021), who also used mice in wound healing.

This research was conducted using only the methanolic root extraction process of *S. jamaicensis* (Truong et al. 2019), as this method was shown to provide the percentage yield in various extractions. This study compared the natural wound healing effects of *S. jamaicensis* in two different concentrations (5% and 10% concentrations).

➢ *Definition of Terms*

The following terms are operationally defined for clarity and a better understanding of the study:

- Excision. The surgical removal of a portion of tissue. This is the most commonly employed method model used to study wound healing.
- Flavonoids. Chemical compounds found in many fruits and vegetables that are strong antioxidants, and scavengers of free radicals, being studied for many properties relating to health including anti-inflammatory, wound healing, and protective properties for heart and brain.
- Maceration. A method in the extraction process that involves soaking plant material in a solvent, such as water or alcohol, to dissolve compounds. Maceration is suitable for extracting soluble compounds, but it has a long extraction time and low extraction efficiency.
- Male Swiss albino mice. An inbred type of mouse imported from Switzerland in the 1950's that commonly is used in medical research. They serve as the model for many types of research, and are suited for this study due to their breed consistency.
- Ointment. The topical vehicle that contains the most oil, usually 80% oil and 20% water. It supports rapid re-epithelization of acute wounds, benefits from moist wound healing condition and thus in itself promotes wound healing.
- Petroleum jelly. An ointment generally recommended as a vehicle for moist wound healing because of its safety and moisturizing qualities. It is considered inert, no active ingredients, and is organic, formed from mineral oils and waxes.
- Povidone iodine. A complex of iodine and polyvinylpyrrolidone (PVP) that releases iodine slowly when it comes into contact with tissue. When used in dressings or as a topical agent, shown to have a benefit on time to wound healing, due to its antimicrobial properties. *Stachytarpeta jamaicensis.* A common plant found in rural tropical areas worldwide, including the Philippines, and is one of the folk medicines found to be effective for topical treatment, thought to be due to its antioxidant content.

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- Wound healing. A normal process of the body in response to injury. It happens in four stages, and involves hemostasis, inflammation, proliferation and remodeling .

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CHAPTER TWO
REVIEW OF RELATED LITERATURE

This chapter presents a review of some of the salient current research which undergird this present study. It gives the researchers an overview of areas already explored concerning the potential wound healing properties of the methanolic root extract of *S. jamaicensis.* This information shows a way forward to fill in the gaps of knowledge with this relevant research.

A. Stachytarpheta jamaicensis

➢ *Taxonomy of S. jamaicensis*

According to the CABI database (Center for Agriculture and Bioscience International 2019 Nov 21), the taxonomic classification of *S. jamaicensis* is specified as follows:

- Domain: Eukaryote
- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiospermae
- Class: Dicotyledonae
- Order: Lamiae
- Family: Verbenaceae
- Genus: Stachytarpheta
- Species: *S. jamaicensis*

➢ *Morphology of S. jamaicensis*

Fig 2 *S. jamaicensis.* (Jamaica Vervain. 2022)

S. jamaicensis, figure 2 commonly known as blue porter weed, is familiar in many parts of the tropics or subtropics and it is abundant in the Philippines. This plant grows between 0.6 and 1.2 meters tall, with a dark green, smooth stem that becomes woody around the base. Typically, this plant produces 5-7 mm long blooms that are a mix of pink and bluish, but it can also produce purple or deep blue flowers. It has grayish-green leaves with a smooth surface, elliptic to ovate form, round apex and noticeable petioles. The colorful flower of *S. jamaicensis* is one of the most eye pleasing features. Such small, tubular flowers are mainly arranged in

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shade of blue or violet color on a long, slender spike. The narrow entrance of this five-lobed, five petaled flower attracts such pollinators as bees and butterflies (Yadav et al. 2022).

S. jamaicensis can be cultivated in a variety of tropical environments and will prosper alongside open fields and vacant lots which receive plenty of direct sunlight. Warm wet conditions will also augment soils which tend to be of lower nutrient density. *S. jamaicensis* uses its fibrous root system as a durable anchor to the ground, and its thick leaves help hold moisture within the plant thereby making it relatively resilient in drought or intense sunlight. This species is widespread in the Philippines, particularly in lowlands where the climate is more favorable for its growth. It appears frequently in rural and urban areas, often doing well in disturbed soils of roadsides or empty fields. Its adaptability has led to its wide distribution in the country, making it available for use in traditional medicines (Jacela et al. 2021).

➢ *Traditional Uses of S. jamaicensis*

S. jamaicensis is a plant used in traditional medicine in some parts of the world, particularly in America, Africa, and Asia. Jacela reviewed that in Philippines, there is an abundance of *S. jamaicensis* where locals use it as a poultice in treating open wounds, for its known to have antibacterial properties (Jacela et al. 2021).

Elderly people have also long used *S. jamaicensis* as a stomach cooling tonic. Before being ingested, the plant's leaf and stem extracts are typically made into tea ba gs. This cooling tonic is used to help with digestive issues such indigestion, acid re flux, ulcers, constipation, dyspepsia, and poor digestion, or to stimulate the gastrointes tinal tract (Liew and Long 2018).

In addition, it is frequently used to treat cirrhosis, hepatitis, allergies, and respi ratory disorders such bronchitis, cough, cold, flu, and asthma (Okwu et al. 2020).

In Southern Nigeria, *S. jamaicensis* is practiced by women to cure feminine complaints and menstruation issues. After giving delivery, the ladies are given a tea made from boiling leaves in water to help the uterus return to its natura l place in the body. In nursing women, it is also known to enhance milk production and control hormones (Okwu et al. 2020).

➢ *Pharmacologic Activities of S. jamaicensis*

Recent studies have brought to the fore reasons why *S. jamaicensis* could be useful for such traditional applications. It was reviewed that S. *jamaicensis* leaves and seeds was found to contain, potential of flavonoids, that alter the four phases of wound healing via different mechanisms has been investigated and deserves further work (Zulkefli et al. 2020). Terpenoids that promotes wound healing primarily due to their astringent and antimicrobial property (Asumang et al. 2021). Tannin that leads to improved wound healing and reduced scar tissue formation by inhibition of the formation and removal of reactive oxygen substances (Chokhoto et al. 2005). Polyphenol in wound healing with its fascinating inherent biocompatible, bio adhesive, antioxidant, and antibacterial properties (Ejiohuo et al. 2024).

➢ *Phytochemical Constituents of S. jamaicensis*

The methanol extract of *S. jamaicensis* leaf prepared were analyzed following several phytochemicals such as flavonoids, phenols, tannins and terpenoids which possess antimicrobial, anti-inflammatory and antioxidant properties (Caluya 2017).

Table 1 below, summarizing the phytochemical content of *S. jamaicensis* (Caluya 2017).

Table 1 Summary of phytochemical constituents present in *S. jamaicensis*

Phytochemical Composition	**Absence/Presence**
Flavonoids -flavonols	+
Phenols	+
Tannins- condensed	+
Terpenoids	+

- *Flavonoids*

The Malaysian team of researchers, led by Nabilah Zulkefli wrote an excellent review of the wound healing properties of flavonoids. They write in their abstract, “Numerous studies have reported that flavonoids possess wound-healing properties due to their well-acclaimed anti-inflammatory, angiogenesis (growing blood vessels), epithelialization (growing new skin), and antioxidant effects.” Summarizing their detailed biological explanations, it seems flavonoids are active in all four stages of wound healing, by numerous pathways detailed by the author. As far as mechanism, flavonoids have been found to be potent reactive oxygen species (ROS) inhibitors, which gives them their antioxidant, anti-inflammatory and antibacterial action in the cell and surrounding injury site. The most studied flavonoid is quercetin, but many others have been found to have similar potential (Zulkefli et al. 2020). Quercetin is a plant pigment (flavonoid) that people sometimes take as a medicine. Flavonoids are antioxidants that neutralize and help avoid damage from free radicals. Free radicals are particles in your body that harm and even kill cells and cell membranes (Stuart et al. 2024).

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- *Phenols*

S. jamaicensis phenols have anti-inflammatory, antibacterial, and antioxidant qualities that make them important for wound healing. These phytochemicals aid in the reduction of bacterial infection and oxidative stress at the wound site, both of which are essential for quicker tissue regeneration and healing. Furthermore, phenols aid in the regulation of inflammatory reactions, which accelerates the development of scabs and epithelialization (Caluya 2017).

- *Tannins*

According to Soni et al. (2021), tannins have antibacterial and astringent properties that help them scavenge free radicals and aid in wound healing. They are also known to have antioxidant activity, which helps them prevent oxidative damage to tissues and facilitate wound healing (Akanji and Sonibare 2018).

- *Terpenoids*

Terpenoids contribute significantly to wound healing through various mechanisms. One of the key actions is their anti-inflammatory effect, as demonstrated by limonin, a triterpenoid compound. Limonin reduces inflammation by inhibiting proinflammatory cytokines, which are crucial during the early phase of wound healing, ensuring an optimal environment for tissue repair (Usman et al. 2024).

B. The Physiology of Wounds and their Healing

Manna Biagio and team detailed the natural organized sequence of four overlapping phases that result in this tissue reconstitution. This process involves hemostasis, inflammation, proliferation, and ultimately the formation of mature scar tissue. Healing begins immediately after the injury, as inflammatory cells migrate to the wound site following platelet activation during the first several hours. The proliferative phase occurs 3 to 21 days after the injury and involves the processes of angiogenesis, granulation tissue production, collagen deposition, and epithelialization.

The primary outcome of this phase is the closure of the wound. Wound healing is to restore the protective epithelial barrier. Without this defense, our initial protection is gone, which leaves us vulnerable to outside pathogens and fluid loss. Wound healing is important for regaining tissue volume and strength (Biagio and Hailey 2023).

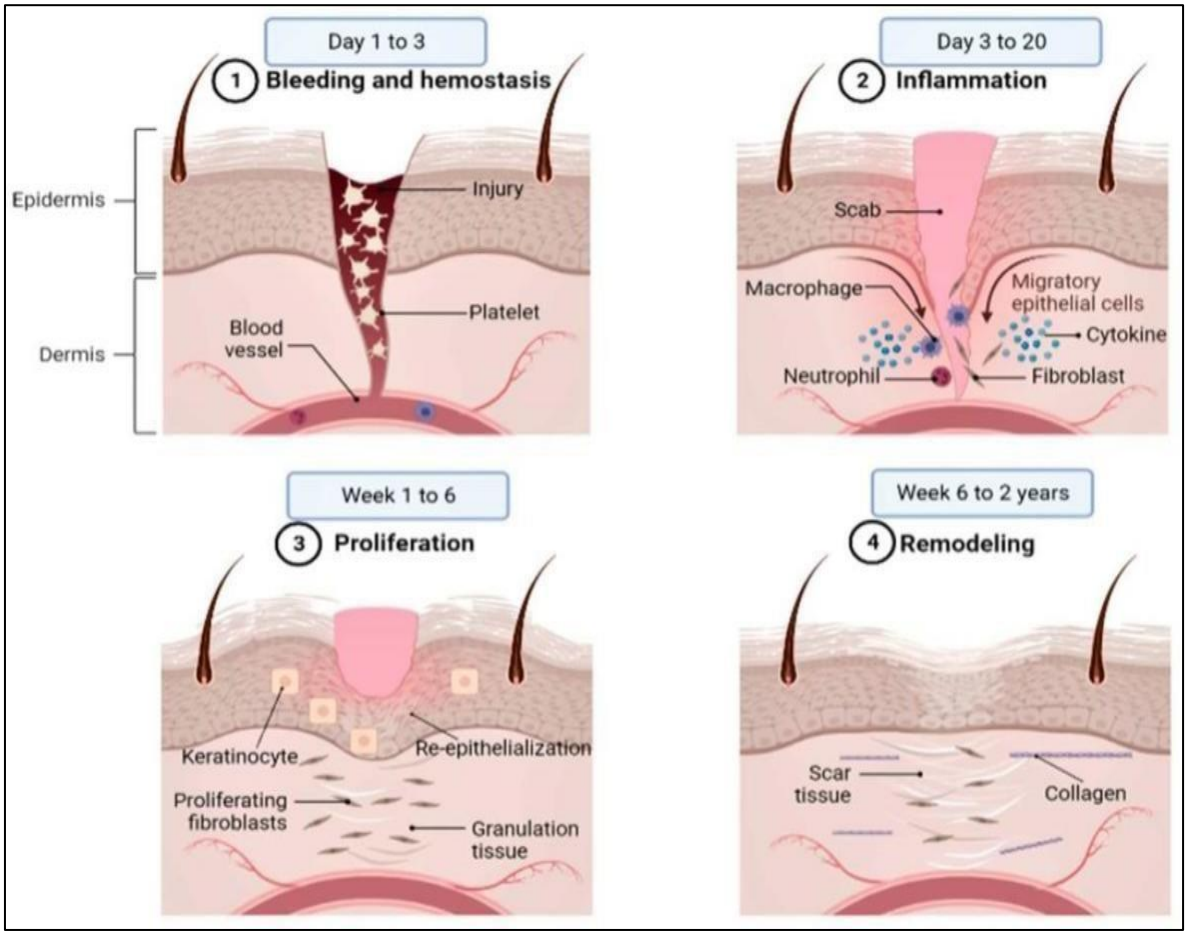

Fig 3 The Physiology of Wounds and their Healing (Biagio and Hailey. 2023)

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C. Wound Excision Model

The excision wound model was one of the standard models used when studying wound healing. In this model, the wound was introduced by removing some tissue, allowing researchers to observe and measure the healing process following the injury. The depth of the wound could vary from superficial to full thickness. In the areas of wound contraction, the regeneration of the skin provided much-needed insight into how wounds healed and also offered a better understanding of the mechanism of healing in wounds. Excision wounds had been widely used in different studies on animal subjects to explore the mechanisms of wound healing in them (Masson-Meyers 2020).

D. Medications for Wound Healing: Povidone Iodine

It was found that Povidone iodine was a very popular topical antiseptic preparation used for the treatment and prevention of wound infections. Its main reasons for effectiveness were its wide spectrum of antibacterial activity, the possibility of biofilm penetration, the absence of resistance development, and its anti-inflammatory properties. In clinical practice, it was not demonstrated to adversely affect the healing of wounds but rather to improve this process (Bigliardi et al. 2017).

Wounds could be made smaller and healed faster with proper chemical solutions in the wound. Povidone-iodine had been well used as an antiseptic agent, and because of its effectiveness in the prevention of wound infection and its potent healing speed, it was believed that more studies would focus on the agent in the near future. Some studies had shown that povidone iodine mixed with saline was more effective than saline solutions alone in significantly reducing surgical site infection and appeared to be potentially more effective than hypochlorous acid in enhancing the healing rate of a wound (Harun et al. 2024).

E. Ointment

(Cherney and Timmons 2022) It produced a barrier for the wound from bacteria. Lastly, it was easy to apply for wound healing. They were semisolid preparations applied to the skin that usually had an oily or greasy consistency and could feel stiff when applied to the skin.

It was used for a variety of conditions for medical purposes, such as cuts (Kuhlmann et al. 2019).

F. Theoretical Framework Feedback

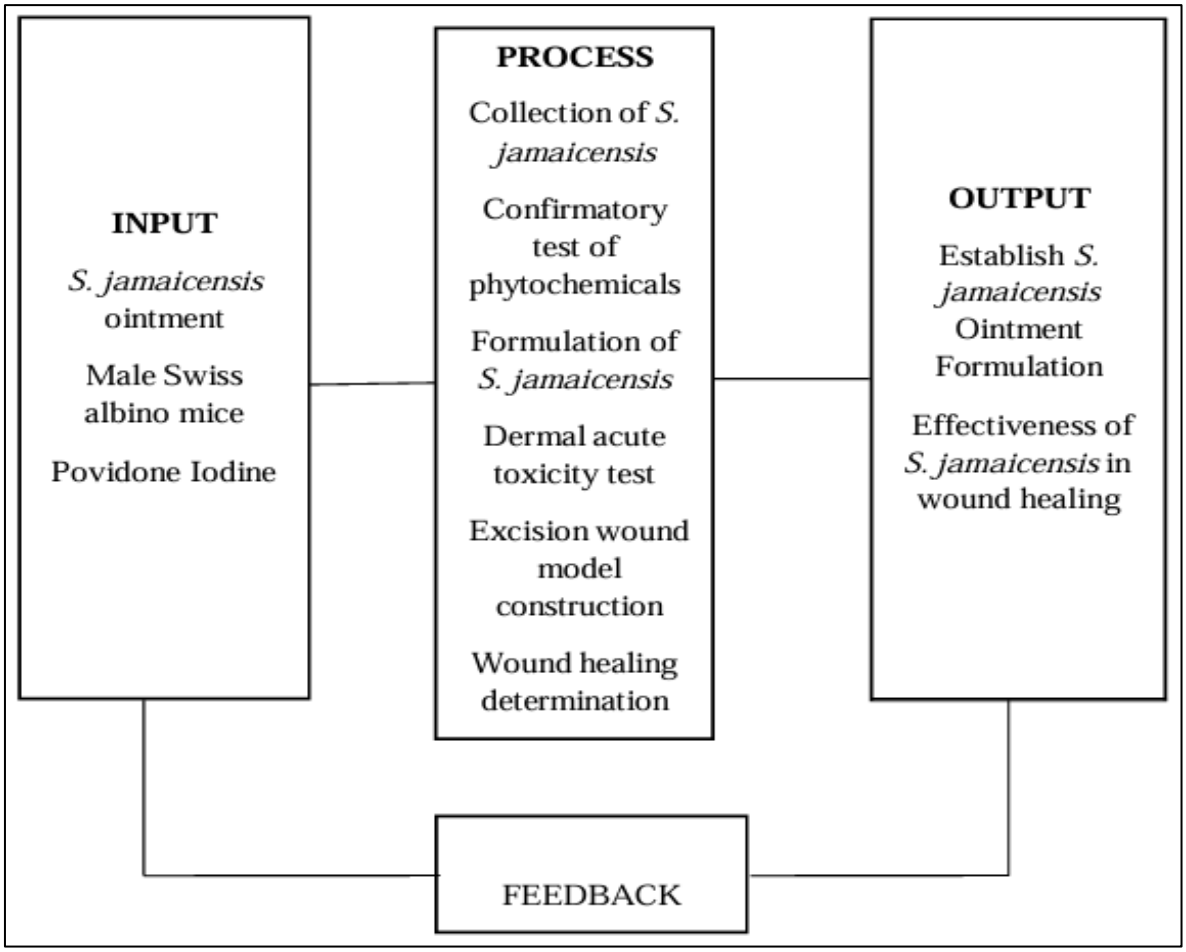

Fig 4 Theoretical Framework

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This was the theoretical framework that provided to the comprehensive understanding of wound healing by *S. jamaicensis*. This research was used an input, process, and output framework in analyzing the complex relationship of elements in the study. The input considers *S. jamaicensis* ointment as a potential agent for wound healing, the Male Swiss albino mice serving as the experimental animal, and the use of povidone iodine as positive control. The process includes various procedures such as the collection of *S. jamaicensis*, Collection of *S. jamaicensis*, Confirmatory test of phytochemicals, Formulation of *S. jamaicensis*, Dermal acute toxicity test, Excision wound model construction and Wound healing determination. The output is designed to establish *S. jamaicensis* Ointment Formulation and Effectiveness of *S. jamaicensis* in wound healing. This framework thus provides a systematic approach to investigating the potential of *S. jamaicensis* as a wound healing agent. Related Studies.

G. White Petrolatum as Negative Control

Petrolatum is used as a negative control due to its inert nature, protective barrier function, and noncomedogenic properties. It ensures accurate assessment of other treatments' effects without interference (Kamrani et al. 2024).

H. Crude Extract Using 5% and 10% Concentration

Ointment formulation using 5% (w/w) and 10% (w/w) with 80% methanol induced significant ($P<0.05$) improvement in wound contraction rate, epithelialization time and skin breaking strength in excision, incision (Giorgis et al. 2022).

I. Ointment as Application

The wound healing ointment exhibited excellent local tolerability with superior assessments in comparison to treatment utilizing only dressings without ointment (Khulmann et al. 2019).

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CHAPTER THREE
METHODOLOGY

The methodology of this research study was driven by the major question: Did the ointment made from the extract of *S. jamaicensis* root improve wound healing? The theoretical framework guided the study toward its conclusions. This chapter continued the discussion of the methodology used in exploring the extract of *S. jamaicensis* for its potential wound-healing activity. The research included various steps, such as identification and authentication, collection and preparation of the plant material, testing the preparation in animals, and analyzing the outcome.

A. General Procedure

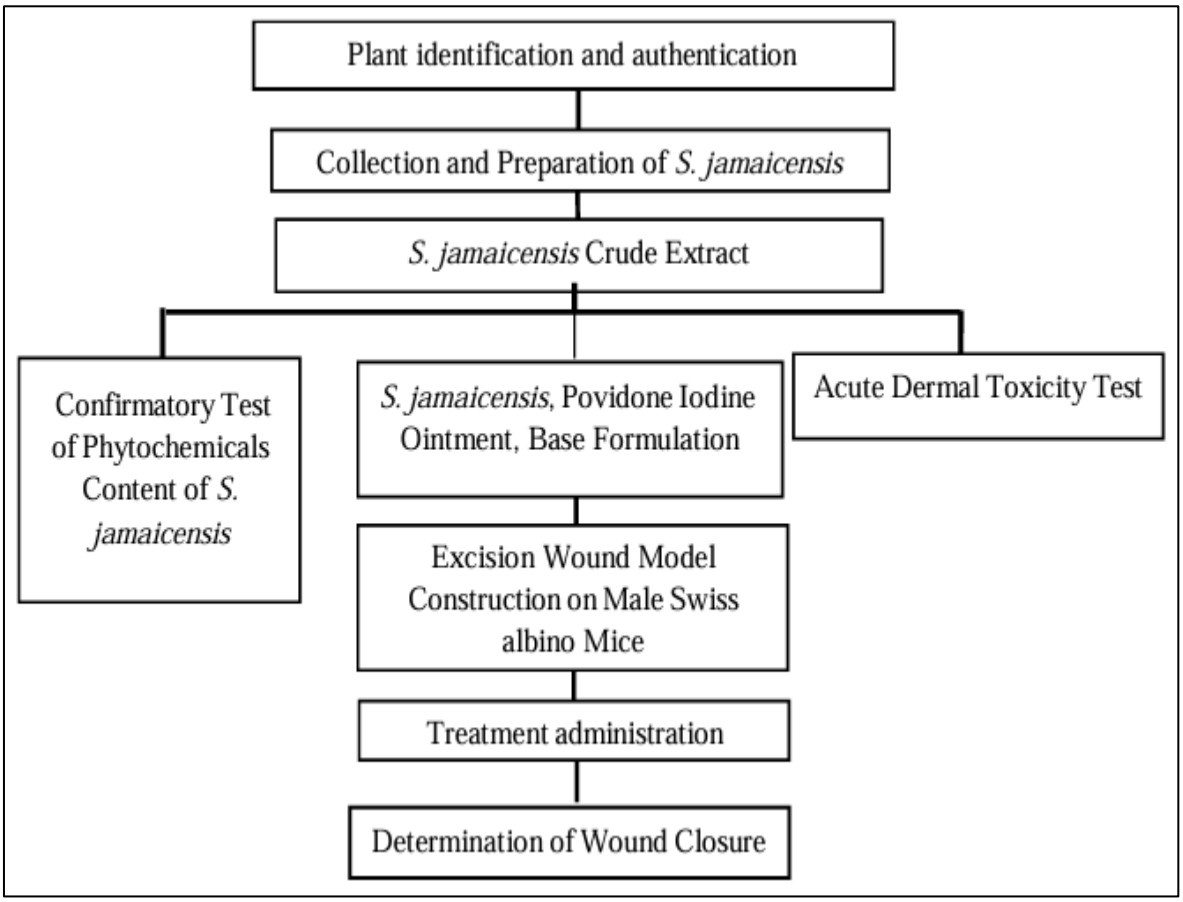

Fig 5 Flow Chart

B. Plant Collection and Extraction

➢ *Collection of S. jamaicensis*

Samples of *S. jamaicensis* were identified and collected in the month of December from the field area of Sitio Dalem at Barangay Barorao, Balabagan, Lanao del Sur. The species were validated for accuracy by an experienced botanist from MSU-IIT.

➢ *Authentication of S. jamaicensis*

The plant authentication was performed by a plant biologist at the Department of Biological Sciences, College of Science and Mathematics, Mindanao State University – Iligan Institute of Technology, Tibanga, Iligan City (8.242455042996502° N, 124.24449592464302° E) to ensure accuracy and reliaility in the identification process.

➢ *Crude Drug Preparation and Extraction of S. jamaicensis*

The collected roots were cleaned thoroughly using tap water to dislodge any soil or debris attached. Then, the roots were allowed to air-dry at room temperature (range of 15-25°C) for 3-6 weeks. The roots were then ground into a powder using a food processor (Alemu et al. 2020).

After grinding, the dried root of *S. jamaicensis* (weighing approximately 185g) was macerated with 1,850ml of 80% methanol, divided evenly into eight 250ml Erlenmeyer flasks, for 72 hours with regular shaking using an orbital shaker (range of 50-300 rpm)

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throughout this period. At a ratio of 1:10, this produced 10.5g crude roots extract (Sari et al. 2022). After filtering the suspension on filter paper (Whatman No.1).

Calculating the need for a minimum of 0.1mL of ointment per mouse once a day at 8A.M in the morning in 5% and 10% concentrations, this process provided more than the estimated required 4.5g of pure extract. The extract was stored in an ointment container and kept in a refrigerator until it solidified. The extract was then poured into a clean ointment jar and labeled for identification. (Alemu et al. 2020).

The researcher performed triplicate analysis to calculate the percentage yield. The crude extraction was conducted three times using 20g of powdered root of *S. jamaicensis* macerated with methanol as the solvent. The weight of the crude extract was divided by the weight of the dried roots sample and multiplied by 100%. The average percentage yield of the crude methanolic extract was determined using the formula given below:

$$\%\ (\text{w/w})\ \text{yield} = \frac{weight\ of\ crude\ extract\ (g)}{weight\ of\ dried\ root\ sample\ (g)}\ x100$$

- *Confirmatory Test*

Confirmatory tests in the analysis of phytochemistry were an essential step toward the verification of the presence of certain types of compounds in plants. These confirmatory tests followed the primary screening and involved reactions with special reagents that produced clear visual clues, such as color change or precipitation. Thus, confirmatory tests tended to confirm the presence of a given class of phytochemicals more certainly than tests at the screening level (Shaikh and Patil 2020).

Table 2 below provided an overview of standard phytochemical tests used to identify different bioactive compounds within plant extracts. Each row focused on a single class of phytochemicals, such as flavonoids, tannins, phenols, and terpenoids. The test procedure, expected results, and references for each were included in the table. This was important for those conducting phytochemical studies on plant materials, as it gave a systematic means of identifying and characterizing the bioactive compounds of interest.

Table 2 Procedures for the Confirmatory Test

Phytochemicals	Test	Procedure	Result	References
Flavonoids Mode of Action: Flavonoids possess wound-healing properties due to their antiinflammatory, angiogenesis, reepithelialization, and antioxidant effects. They act on the wound-healing process via the expression of biomarkers respective to the pathways.	Alkaline Reagent Test	Add 2-3 drops of Sodium hyrodixide to 2mL of root extract and add dilute hydrochloride.	Deep yellow color and colorless after adding hydrochloride.	Kancherla N, Dhakshinam oothi A, Chitra K, Komaram RB. 2019. Preliminary Analysis of Phytoconstit uents and Evaluation of Anthelminth ic Property of Cayratia auriculata (In Vitro). Maedica (Bucur). 350-356. doi: 10.26574/m aedica.2019. 14.4.350
Tannins	Ferric Chloride Test	Add 2mL of 5% ferric chloride.	Greenish black or Dark blue.	Hambali Dauda, Garba Uba,
Mode of Action: Tannin leads to improved wound healing and reduced scar tissue formation by inhibiting the formation and removal of reactive oxygen substances. It also offers advantages like pain relief, limitation of secondary infection, prevention of plasma loss, and promotion of prolific epithelialization.				and Umar Ali (2020). Preliminary Phytochemic al Screening, Quantitative Analysis of Flavonoids from the Stem Bark Extract of Commiphor a Africana (Burseraceae). BULLETIN OF ENVIRON MENTAL SCIENCE & SUSTAINA

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Phenol Mode of Action: Polyphenols are versatile green phytochemicals with inherent biocompatible, bioadhesive, antioxidant, and antibacterial properties, vital for effective wound dressings that necessitate multifunctional therapeutic substances for rapid wound healing.	Lead Acetate Test	Add 0.5mL of 1% lead acetate solution.	Formation of percipitate.	Deshmukh MA, Theng MA. 2018. Phytochemic al screening, quantitative analysis of primary and secondary metabolites of Acacia arabica bark. International Journal of Current Pharmaceuti cal Research.10 (2):35. doi:10.2215 9/ijcpr.2018 v10i2.25889
Terpenoids Mode of Action: Terpenoids are reported to promote wound healing primarily due to their astringent and antimicrobial properties, which can be responsible for wound contraction and an improved rate of epithelialization.	Salkowski Test	5mL extract will be mixed with 2mL chloroform and add 3mL of concentration H^2SO^4.	Reddish brown color	Dahanayake JM, Perera PK, Galappatty P, Melshandi Perera HD, Arawwawal a LD. 2019. Comparative phytochemic al analysis and antioxidant activities of TAMALAK YADI decoction with its modified dosage forms. Evidence-Based Complement ary and Alternative Medicine.1–9. doi:10.1155/ 2019/60371 37

C. In Vivo Evaluation Using Experimental Animals

➢ *Selection of Animal Species*

Approximately 95% of all animal models in research were done with rats or mice, the majority being inbred albino mice (Gad 2014). Their rapid maturation and fast reproduction facilitated age-associated research and reduced the variability in experiments. The selection of inbred albino mice for this research was based on their genetic and biological consistency, as well as their ease of handling and maintenance, which enhanced the reliability of data collection. The use of albino mice also helped to minimize the ethical dilemma of involving humans in the early stages of experimental research, providing a balance between scientific progress and ethical responsibility (Pracheta et al. 2022).

Male mice were often preferred over females in such studies because their hormonal levels were more stable, whereas fluctuating hormone levels in females, influenced by the estrous cycle, could introduce variability and complicate data interpretation (Zeng et al. 2023).

Twenty male Swiss albino mice weighing approximately 20-30 g and 6-8 weeks of age were procured from Adventist Medical Center College of Pharmacy's Animal House. Only males were chosen because it had been observed that males and females healed at different rates, and female healing rates were more unpredictable, probably due to hormonal changes.

This removed this possible confounding variable (Bui et al. 2023).

➢ *Housing and Feeding Conditions*

The mice were kept in single cages in transparent plastic storage buckets with lids, designed for general use, featuring ventilation and a 1000 mL capacity, with approximate dimensions of 165-millimeter diameter. The separate cages prevented injury from potentially aggressive behavior among male mice (Kasza et al. 2023). The mice remained under normal environmental

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conditions with a temperature range of 20-27°C and humidity of 82 ± 10% (both typical for a non-air-conditioned room in the Philippines). These conditions were monitored using a room thermometer and a hygrometer. The mice were provided with an adequate supply of commercial mouse pellets. Since the average mouse eats 5g of food per day, multiplying by 20 mice and 30 days, 15 kg of feed was purchased and provided for ad libitum of 21L. They were given a week to acclimatize before the study began. They had access to adequate clean drinking water. Research shows that the average mouse drinks 5-7ml per day, possibly triple that in hot weather. Thus, a small hanging bottle for water, and dish for pellets were placed in each cage, with the water container holding no less than 30mL. Normal room standard conditions were maintained with a 12-hour light/dark cycle This was as per recommended in the research of (Schroder et al. 2023).

D. Preparation of the Test Substances

➢ *Preparation of Ointment from the Extract*

To prepare the *S. jamaicensis* extract ointment, petroleum jelly was use as a base and *S. jamaicensis* methanolic root extract were combined at the two concentrations of 5% and 10%.

Based on the research of Allen (2020), an ideal ointment base should be stable, smooth, compatible with the skin, non-irritating, and should release the incorporated medicaments readily. This study employed an oleaginous specifically, petroleum jelly which is lipophilic ointment, immiscible with water, avoiding moisture evaporation, and can remain on the skin for a long period of time. The adsorption base can adsorb water and is difficult to remove through washing (Renukuntla et al. 2022).

In this preparation, the Oleaginous Base (Petroleum jelly) was first melted in a water bath. Once fully liquefied, the appropriate quantity of *S. jamaicensis* was added to achieve the desired concentrations. For the 5% formulation, 1.5g of the plant extract was added to 28.5g of petroleum jelly. For 10% formulation, 3g of extract was combined with 27g of petroleum jelly. The mixture was stirred continuously while still warm until a uniform consistency was achieved. The homogeneous mixture was then poured into sterile ointment jars and left uncovered at room temperature to solidify. Once the ointment had congealed, jars were sealed and labeled accordingly. This procedure was done following the pattern given in the research of (Yiblet et al. 2022).

Table 3 below outlined the ingredients and proportions used in the formulation of the ointment for each of the 5% and 10% ointments, using two concentrations: Povidone Iodine (positive control) and Oleaginous Base (negative control).

Table 3 Ingredients and Proportions of Test Substances

GROUP OF MICE	Ointment to be applied:	10% Povidone Iodine	Petroleum Jelly	*S. jamaicensis* Extract
Group 1	5% *S. jamaicensis* extract ointment		28.5 grams	1.5 grams
Group 2	10%*S. jamaicensis* extract ointment		27 grams	3 grams
Group 3	10% Povidone Iodine ointment (positive control)	30 grams		
Group 4	Petroleum Jelly (negative control)		100grams	

➢ *Preparation of the Positive Control (Povidone Iodine)*

Povidone-iodine, especially the 10% solution, has extensive documentation regarding its efficacy in facilitating wound healing. From earlier studies, the formula established its effectiveness in preventing infection and aiding granulation tissue formation in the inflammatory stage (Raziyeva et al. 2021).

The preparation method of povidone iodine ointment involved heating of 7.5g polyethylene glycol-400 and 7.5g of PEG-4000 with stirring at 78-82°C for 30 minutes. Then, 3g of xylitol was added to this mixture and stirred for 15 minutes at the same temperature. Separately, 0.3g of sodium lauryl sulfate was dissolved in 3.51g of purified water at 78-82°C while stirring for 15 minutes. The contents of the two containers were then blended together in a blender and stirred for another 15 minutes at 78–82°C. The mixture was then cooled to 60–65°C, followed by mixing with 0.09g of potassium iodate solution and 20% povidone iodine powder. Finally, the mixture was cooled, degassed, and stored in an ointment jar (Wuhan Diao Pharmaceutical Co. Ltd. 2023).

Table 4 below provides the composition and amount of ingredients that were used for the preparation of positive control solutions for the 10% povidone-iodine ointment as follows: 10% povidone iodine, 24% polyethylene glycol-4000, and 20% glycerol. This was gleaned from the formulary of Wuhan Diao Pharmaceutical Co. Ltd. (2023).

Table 4 below listed the required measurements for the preparation of the ointment with preferred properties. The constituents were mixed in a particular ratio to produce the desired consistency and effectiveness of the ointment.

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Table 4 Ingredients and Quantity of the 10% Povidone Iodine Ointment.
(Povidone iodine Ointment and Preparation method 2014).

Ingredients	Functions	Quantity
Povidone iodine	Active ingredient	3g
Potassium Iodate	Iodine deficiency	0.09g
PEG-400	Thickening agent and Base	7.5g
PEG-4000	Thickening agent and Base	7.5g
Xylitol	Humectant Skin soothing	3g
Glycerol	Humectant Enhancing moisture skin retention soothing	5.1g
Sodium lauryl sulfate	Surfactant Ensuring consistent distribution of the active ingredient	0.3g
Purified water	Solvent It also helps to hydrate the skin	3.51g

➢ *Preparation of Negative Control (White Ointment)*

Table 5 Ingredients for Oleaginous Base (Shrewsbury 2024).

White Wax	5%	5g
White Petrolatum	95%	95g

- *Procedure for Preparation:*

✓ The white wax was melted on a hot plate, but not beyond 70–75°C.
✓ When the wax had completely melted, the petrolatum was added, and the entire mixture was allowed to remain on the hot plate until liquefied.
✓ Following liquefaction, the mixture was removed from heat and allowed to congeal. The mixture was stirred until it began to congeal.

E. OECD 402: Acute Dermal Toxicity

Before beginning the wound healing experiment, the methanolic root extract of *S. jamaicensis* was tested for acute dermal toxicity by following a simplified version of the OECD 402 guidelines. These guidelines are revised every 25 years, most recently in 2017. The intent of the test was to determine the safe range of concentration of the extract to use in the research, to affirm or adjust the presumed concentrations of 5% and 10%.

➢ *Administration of the Test Substance for Acute Dermal Toxicity*

To assess the dermal toxicity of the methanolic root extract of *S. jamaicensis*, researchers tested four dosage levels, controlled group (50mg/kg), low dose (200 mg/kg), medium dose (1000 mg/kg), and high dose (2000 mg/kg) using ointment formulations. Each dosage group included three mice, totaling 12 mice for the study. Every mouse had one wounds on the center treated with the ointment. Formulations contained equal concentrations of the extract to ensure uniform dosing and facilitate a direct comparison. To prevent the mice from licking or scratching the application sites and to ensure proper absorption, Elizabethan collars were fitted for 24 hours post-application. This setup allowed for the observation of any immediate skin reactions such as irritation or inflammation. The mice were then observed over a 14-day period for both local and systemic effects, including skin reactions like redness or swelling and general health indicators such as behavioral changes, body weight, and food and water intake.

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➢ *Reading of Acute Dermal Toxicity Test*

Table 6 Checklist of Reading the Acute Dermal Toxicity Test

First Batch ... Trial with 5% preparation:		DAY 0: record time of application: _____					DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
Mouse 1	write yes or no:	30 minutes	1 hour	2 hours	4 hours	6 hours	8AM	8AM	8AM	8AM	8AM	8AM
Skin:	Redness											
	Blisters											
	Scratching											
	fur loss											
Mucous Membranes:	Eyes red or draining											
	drooling											
Breathing patterns:	gaping mouth											
	panting											
Nervous system:	agitation											
	tremors											
	seizures											
	oversensitive to touch											
Mouse 2	write yes or no:											
Skin:	Redness											
	Blisters											
	Scratching											
	fur loss											
Mucous Membranes:	Eyes red or draining											
	drooling											
Breathing patterns:	gaping mouth											
	panting											
Nervous system:	agitation											
	tremors											
	seizures											
	oversensitive to touch											

The mice were observed immediately after dosing, then at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 2 to 6 hours after the beginning of the exposure period, and daily thereafter, for a total of 14 days. Reactions to watch for included changes in skin like redness or blisters, itching behavior, and changes in fur, eyes, and mucous membranes (OECD 402). A checklist was placed at the cage of each mouse to allow observers to note any of the above reactions. This way, careful records were kept of any reactions.

The results are placed in Chapter 4, and in appendix O was carried out again following the prior example of previous researchers, namely, Belachew TF et al. 2020.

F. Excision Wound Model

Twenty mice were chosen for this wound healing experiment and divided into four groups as noted above in 3.4, Table 3.

➢ *Creation of an Excisional Wound*

Researcher performed hair removal by applying Veet cream at least 1-2 minutes at dorsum site and anesthetized using topical anesthesia (Lidocaine ointment USP, 5%). The site of the wound was wiped with a piece of cotton and 70% ethanol to sterilize the area (Yiblet et al. 2022). A small amount of skin was excised using a sterile disposable skin punch biopsy with an inside diameter of 8 mm at the dorsum site. The size of the wound was thus 8 mm circular, and the depth was 0.5 mm (Hora et al. 2024).

Before treatment was begun, the mouse was monitored until fully awake from anesthesia and hemostasis (stopping of bleeding) was obtained.

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Fig 6 The Location of wound to be Excised is on the Dorsum Site of the Mice (Zhang et al. 2023).

G. Administration of the Test Substances

The experimental mice were divided into four groups, with each group consisting of 5 members. The first group was treated with methanolic root extract of *S. jamaicensis* 5% ointment. The second group was treated with the extract ointment of 10%. The third group was treated with Povidone iodine 10% ointment as the positive control. The fourth group, as the negative control group, was treated with only pure petroleum jelly.

Table 7 Study Design for the Administration of Test Substance

Group Label	Treatment Method
Group 1	5% methanol extract of *S. jamaicensis*
Group 2	10% methanol extract of *S. jamaicensis*
Group 3	Povidone iodine 10% ointment.
Group 4	Negative control

Once daily, for 14 days, or until the wound was completely closed, these different concentrations of ointment were applied. The 5% methanolic crude root extract of *S. jamaicensis* represented the low concentration from the result of the acute dermal toxicity test, while the 10% Povidone ointment was used as the high concentration of 10% Povidone iodine. Approximately 0.1 mL was applied using sterile gloves, evenly on the wounds, measured by a tuberculin syringe without a needle (Bezu et al. 2021).

H. Data Collection

The researchers kept a careful chart record of each daily measurement of wound diameters, with one person assigned to transfer the data to an Excel spreadsheet. The healing progress was measured for 14 days, and the wound area was measured on days 0, 3, 7, and 14 (Hora et al. 2024).

A caliper was used to measure the transverse (b) and longitudinal (a) diameters, calculating the average radius r (1/4 the sum of the 2 diameters) and then calculating the area (as WA) by the formula Wound Area = (pi)r^2. To calculate the percentage of wound contraction, the researcher used the following formula (Ahmed et al. 2022).

$$\% \text{ wound contraction} = \frac{WA0 - WAt}{W0}(100)$$

Where WA0 = wound area at day 1, WAt = wound area at day T

Where T = number of days

Formula for wound area $A = \pi \left(\frac{a+b}{4}\right) \left(\frac{a+b}{4}\right)$

Where a is longitudinal diameter, and b is transverse diameter.

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I. Statistical Analysis

Recorded data were given as the mean standard deviation (SD), and the study was statistically analyzed using one-way ANOVA to compare the potential healing activity of *S. jamaicensis* root ointment on an excised cutaneous wound on male albino mice. A probability testing of $P<0.05$ was considered to be the significant level.

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CHAPTER FOUR
RESULTS AND DISCUSSIONS

This chapter presents the findings obtained from the crude methanolic root extracts of *Stachytarpheta jamaicensis* (commonly known as Gervao). The analysis includes its average percentage yield, phytochemical composition, acute dermal toxicity assessment of the methanolic root extracts of *S. jamaicensis* (Gervao) and its potential wound-healing properties on excised cutaneous wounds in male albino mice. It also includes the formulation of ointment for wound healing. Relevant discussion and findings are provided.

➢ *Preparation of Plant Extract*

The crude methanolic root extracts of *S. jamaicensis* (Gervao) appear blackish-brown color, with a semi-solid, pasty consistency at the bottom, emitting a scent similar to coffee bean.

Fig 7 The Crude Methanolic Root Extract of *S. jamaicensis* (Gervao)

The extraction process resulted in an average yield of 4.5%. From the 60 grams of extract, it was divided into 3 equal parts. Table 8 provides a detailed breakdown of individual yield results from each trial.

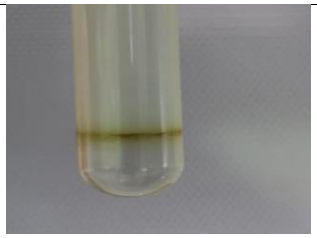
Table 8 Percentage Yield of the Methanolic Root Extract of *S. jamaicensis* (Gervao)

Trials	Weight of Dried Roots	Weight of Crude Extract	Percentage Yield
1	20g	1.1g	5.5%
2	20g	0.9g	4.5%
3	20g	0.7g	3.5%
		MEAN VALUE	4.5%

➢ *Phytochemical Confirmation Test of S. jamaicensis*

The result of the phytochemical screening test of plant constituents presents in the crude methanolic root extract of *S. jamaicensis*. The methanolic root extract of *S. jamaicensis* was found to contain flavonoids, phenols, tannins, and terpenoids.

Table 9 Confirmatory Test for the Presence of Flavonoids, Phenols, Tannins, and Terpenoids of the Methanolic Root Extract of *S. jamaicensis*.

Test	Visible Results	Interpretations
Flavonoids (Ferric Chloride Test)	 A colorless color was observed.	Presence of Flavonoids

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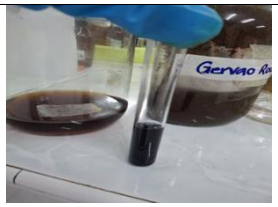
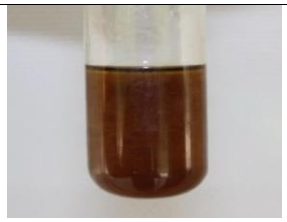
Phenols (Lead Acetate Test)	 A formation of precipitate was observed	Presence of Phenols
Tannins (Ferric Chloride Test)	 A greenish black color was observed	Presence of Tannins (Condensed Tannins)
Terpenoids (Salkowski Test)	 A reddish-brown color was observed	Presence of Terpenoids

➢ *Acute Dermal Toxicity - Fixed Dose Procedure*

Acute dermal toxicity was assessed using doses of 50, 200, 1000, and 2000 mg/kg. At the lowest dose of 50 mg/kg, there were no observable behavioral changes, signs of toxicity, or deaths. Similarly, mice treated with 200 mg/kg exhibited no toxic effects or unusual behaviors. At 1000 mg/kg, there were no instances of death, convulsions, tremors, or behavioral abnormalities. The highest dose of 2000 mg/kg also resulted in no mortality, seizures, tremors, or abnormal behaviors. Throughout the 14-day observation period, none of the treated mice showed signs of systemic toxicity or any notable physical or behavioral changes.

➢ *Formulation of S. jamaicensis Ointment*

In the ointment formulation, petroleum jelly acts as the primary base, with crude root extract of *S. jamaicensis* added at concentrations of 5% and 10% (Yiblet et al.2022). The formulation is developed to maintain a consistent texture and ensure even distribution of the active extract throughout the base. Petroleum jelly not only provides a semi-solid structure but also contributes occlusive effects, which are advantageous for promoting wound healing. The extract concentrations are calculated using the weight-to-weight (w/w) method, indicating the proportion of extract relative to the total weight of the formulation.

Table 10 outlines the components and their respective quantities used in the ointment, highlighting the variation in extract amounts according to the concentration levels. The extract, rich in bioactive compounds, serves as the active agent in the formulation, intended to support wound-healing activity.

Table 10 Ingredients and Proportions of the Plant Extract Ointment (5% and 10%)

Ingredients	***5% S. Jamaicensis* Extract**	***10% S. jamaicensis* Extract**
Petrolatum	28.5g	27.0g
S. jamaicensis methanolic root extract	1.5g	3.0g

- *Procedure for 5% and 10% S. jamaicensis Ointment*

✓ For 5% weigh 1.5 g of *S. jamaicensis* extract and 28.5 g of white petrolatum. For 10% weigh 3g of *S. jamaicensis* extract and 27g of petrolatum.
✓ Place the petrolatum in a beaker. Heat gently on a hot plate at 50–60°C until it becomes fully melted.
✓ Gradually add the extract to the melted petrolatum while stirring continuously with a stirring rod
✓ Continue stirring on the hot plate for 5–10 minutes to ensure even dispersion of the extract.
✓ Remove the mixture from the heat. Continue stirring as it cools to maintain uniformity and prevent settling.
✓ Once semi-solid, transfer into a clean, labeled container.

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Fig 8 Finished Product of 5% and 10% S. *jamaicensis*

➢ *Analysis of Variance (ANOVA) Test on the Healing Activity of S. jamaicensis Root Ointment*

This report presents the analysis conducted to compare the healing activity of *S. jamaicensis* root ointment on an excised cutaneous wound on male albino mice. The statistical methods employed for this study were carried out using the R Software.

Table 11 Analysis of Variance (ANOVA) Test on the Healing Activity of *S. jamaicensis* Root Ointment

	DF	**Sum of Sq.**	**Mean Sq.**	**F Value**	***p*-value**
Treatment Group	3	10338	3446	2.931	0.042
Residuals	52	61140	1176		

Legend: $p \leq 0.05$ = Significant; $p > 0.05$ = Not Significant

Table 11 presents the one-way ANOVA test results to assess the effect of different treatments on wound healing using *S. jamaicensis* root ointment.

The results indicate a statistically significant difference in healing percentage among the four treatments as shown in the *p*-value of 0.042 (F = 2.931) which is less than the typical level of significance 0.05. This further suggests that at least one treatment group exhibited a significantly different healing process compared to the others.

➢ *Tukey's HSD Test on S. jamaicensis Root Ointment Groups*

Table 12 Statistical Comparison of Wound Closure Across Treatment Groups

Difference	**Lower Bound**	**Upper Bound**	***p*-value**
Negative Control –27.08 Positive Control	-39.79	29.00	0.17
Negative Control – 29.745% Concentration	-4.65	64.14	0.1123
Negative Control – 35.1410% Concentration	0.74	69.54	0.0436
5% Concentration – -5.39 10% Concentration	-39.79	29.00	0.975
Positive Control – -8.06 10% Concentration	-42.45	26.34	0.925
Positive Control – 5% -2.66 Concentration	-37.06	31.74	0.997

Legend: $p \leq 0.05$ = Significant; $p > 0.05$ = Not Significant

Table 12 presents the results of the Tukey's HSD post-hoc test to compare the wound healing percentage of *S. jamaicensis* root ointment at various concentrations.

Among all comparisons, only the difference between the Negative Control and the 10% concentration was found to be statistically significant with a *p*-value of 0.0436, which is less than the 0.05 significance level. The estimated difference in healing was 35.14 percentage points, and the 95% confidence interval (0.74 to 69.54) does not include zero, supporting the reliability of this finding.

Other treatment comparisons are not found to be significantly different as shown in their *p*values greater than the 5% significance threshold, and their confidence intervals containing zero.

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➢ *Evaluation of Wound Healing Efficacy Based on Wound Area Contraction*

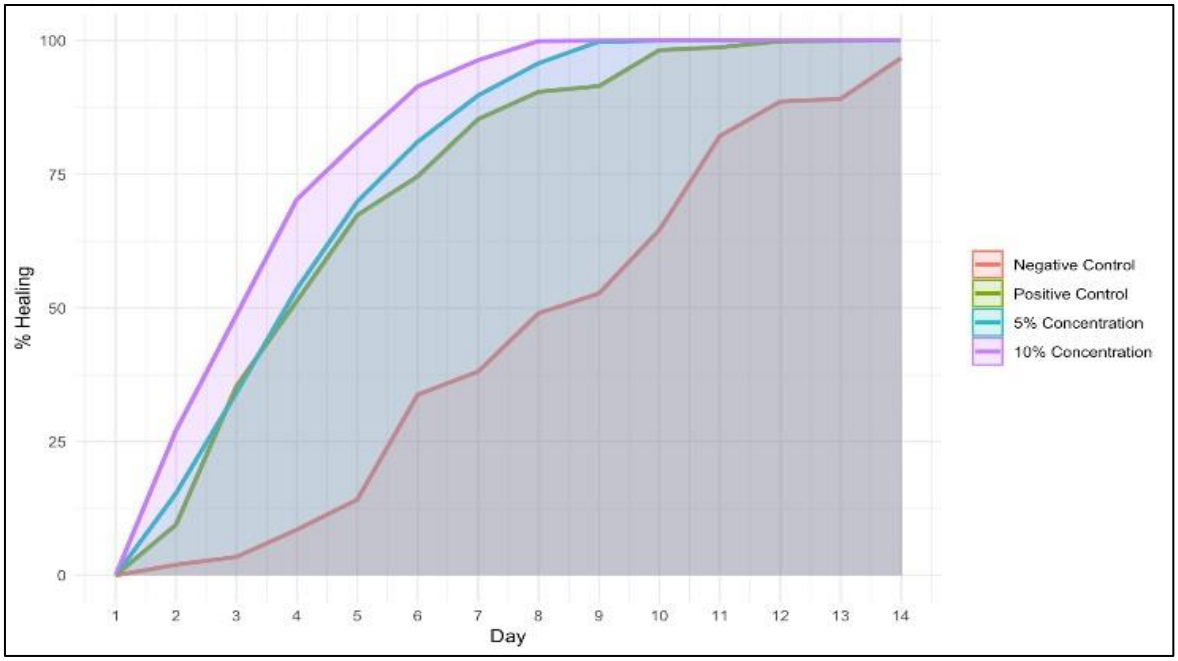

Fig 9 Percent Healing Over a 14-Day Period for Each Treatment Group (Area Under the Curve Representation)

Figure 9 illustrates the healing percentages across different treatment groups applied to male albino mice wounds using *S. jamaicensis* root ointment in an Area Under the Curve representation. It is evident from the graph that the Negative Control group exhibited a slower healing process over the 14-day period. In contrast, the 10% concentration ointment demonstrated a higher percentage of wound healing, suggesting that this treatment was the most effective among the other treatments tested.

➢ *Discussion*

The results support the traditional use of *S. jamaicensis* for wound healing and show the need for scientific validation. One-way ANOVA showed a significant difference between treatment groups ($p = 0.042$), and Tukey's test confirmed that the 10% extract ointment improved healing compared to the negative control ($p = 0.0436$). This effect is likely due to flavonoids, phenols, tannins, and terpenoids in the plant, which are known to help with tissue repair. Flavonoids reduce inflammation and boost blood flow, tannins protect the wound and fight microbes, and terpenoids support new tissue growth (Usman et al. 2024). The 10% extract performed as well as or better than the positive control (Povidone-Iodine), suggesting it could be a low-cost option for wound care. These findings support the plant's traditional use and could lead to new herbal treatments. However, the study was limited to male mice and a 14-day healing period. Future research should test different animals, wound types, and treatment durations, and identify the most active compounds.

This is supported by the literature for wound healing by plant components of flavonoids, phenols, tannins, and terpenoids, which are all contained in *S. jamaicensis*. These phytochemicals are responsible for the increased wound healing with the 10% concentration. Flavonoids, for example, are anti-inflammatory, antioxidant, and pro-angiogenic and are all vital in wound healing. Tannins act by virtue of their astringency and antimicrobial activity, and terpenoids also contribute to tissue repair (Soni et al. 2021).

Many natural products contain compounds that help promote wound healing. Plant-based secondary metabolites such as tannins, flavonoids, phenols, and terpenoids (as shown in Table 9) have been found to support the healing process. Tannins are polyphenolic compounds that form a protective layer over wounds, reducing irritation and aiding recovery.

Phenolic compounds play a key role in wound healing by reducing oxidative stress and preventing infections. They also help stimulate collagen production and support the growth of new tissue. Flavonoids are known for their anti-inflammatory and antioxidant properties. They help skin cells migrate and grow, speeding up wound closure and encouraging the formation of new blood vessels. Terpenoids, especially those found in essential oils, have antimicrobial effects and promote the growth of fibroblasts and collagen, which are essential for tissue repair (Das et al., 2019).

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CHAPTER FIVE
SUMMARY, CONCLUSION, AND RECOMMENDATIONS

This study was performed with the objective of assessing the wound healing potential of methanolic root extract of *Stachytarpheta jamaicensis* as an ointment, in a model of male Swiss albino mice. Statistical analysis for the study was done using R Software.

➢ *Summary of Findings:*

In the present study, the wound healing activity of *Stachytarpheta jamaicensis* root ointment on excised cutaneous wounds in male albino mice was investigated.

- The methanolic crude root extract of *S. jamaicensis* was semi-solid and pasty in texture and with the smell of coffee.
- Phytochemical analysis of the extract qualitatively established the presence of tannins, phenols, terpenoids, and flavonoids.
- The experiment entailed the preparation of an ointment from the *S. jamaicensis* extract and the preparation of a base ointment as a negative control.
- There is no result of toxicity from acute dermal toxicity test of the ointment extract was carried out; it is illustrated from the findings to be the effectiveness of the *S. jamaicensis* extract in healing excised cutaneous wounds in male Swiss albino mice.
- A single-factor ANOVA test identified a statistically significant difference in the percentages of healing between the treatment groups ($p < 0.05$) that showed a general effect of the treatments on wound healing.
- Post-hoc analysis with Tukey's HSD test revealed that the treatment group of the 10% *S.*
- *jamaicensis* root ointment had significantly improved wound healing compared to the negative control group ($p = 0.0436$), evidence for the potential of the extract to close wounds.
- Although a significant effect on wound healing was observed in the 10% concentration, the statistical analysis failed to identify significant differences between the other treatment groups within the experiment, implying a concentration-dependent effect of the extract.

➢ *Conclusion*

The current research investigated the wound-healing activity of crude methanolic root extract of *S. jamaicensis* in male Swiss albino mice. The experiment confirmed that topical application of the extract as an ointment promoted healing of the wound. The extract, a blackish-brown semi-solid with an average of 4.5% (i.e., 4.5% of the dried roots were recovered as crude extract with the compounds of interest), had a significant effect on healing in the treatment groups (ANOVA and Tukey's HSD), with the 10% concentration ointment having the highest and statistically significant effect (ANOVA, $p=0.042$). Groups treated with povidone-iodine preparation and the 5% concentration of *S. jamaicensis* had accelerated healing. *S. jamaicensis*, the highest and only statistically significant wound closure effect was found to be in the 10% ointment concentration.

➢ *Recommendations*

The researchers, based on the results of this study, propose the following for future research:

- Perform acute and chronic toxicity testing of the methanolic root extract of *Stachytarpheta jamaicensis* to establish its safety profile for in vivo application.
- Continue to study the possibilities for clinical use of *Stachytarpheta jamaicensis* extract on human patients with different kinds of wounds.
- Examine the wound-healing activity of other components of the *Stachytarpheta jamaicensis* plant as a potential source of synergistic or alternative therapeutic activity.
- Formulate other pharmaceutical preparations for direct application, e.g., gels and creams, using the *Stachytarpheta jamaicensis* root extract.
- Implement a useful means of objectively quantifying wound size, both length and depth, to improve the accuracy of future wound-healing research.

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APPENDIX A
SCHEDULE OF ACTIVITIES

- Thesis Title: Potential Wound Healing Activity of Methanolic Root Extract of Stachytarpeta jamaicensis (Gervao) Ointment in Male Swiss Albino Mice.

- Project Dates: December 2024 to April 2025

	RESEARCH OBJECTIVES	2024					2025				
		AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY
1	Choosing a Research Topic										
2	Submission of Abstract Proposal										
3	Proposal for Candidate Adviser										
4	Finalization of Research Topic										
5	Writing of Chapter 1: The Problem and Its Background										
6	Writing of Chapter II: Reviews of Related Literature and Studies										
7	Writing of Chapter III: Methodology										
8	Writing of Other Parts of the Proposal Manuscript and Making PowerPoint Presentation for Defense										
9	Proposal Defense Application										
10	Thesis Proposal Hearing										
11	Revision of Proposal Manuscripts According to Thesis Panel Suggestions, Recommendations, and Annotations										
12	Submission of Approved and Signed Proposal Manuscript										
13	Plant Authentication										

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14	Plant Collection										
15	Requesting and Purchasing of Chemicals and Reagents to be										
	Used										
16	Collection and Preparation of ….										
17	Powdering										
18	Extraction										
19	Buying of Mice										
20	Preparation of Test Substances										
21	Administration of Test Substances										
22	Acute Dermal Toxicity Test										
23	Test for wound healing										
24	Documentation/ Statistical Analysis										
25	Finalization of Manuscript										
26	Final defense										

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APPENDIX B
BUDGET PROPOSAL

➢ *Budget Proposal*

- Thesis Title: Potential Wound Healing Activity of Methanolic Root Extract of Stachytarpheta jamaicensis (Gervao) Ointment in Male Swiss Albino Mice.

- Project Dates: December 2024 to April 2025

This Budget Proposal provides necessary costs that is associated with the above named research project. The proposed cost for the research project was itemized below:

EXPENDITURES	BUDGET
Print	PHP 3,000
Phytochemical Analysis	PHP 1,300
Plant Collection and Authentication	PHP 300
Cage	PHP 1,200
Mice	PHP 5,500
Transportation	PHP 5,511.72
Miscellaneous	PHP 2,903
Pharmacy Laboratory fee	PHP 2,000
Chemicals and Reagents	PHP 14,770.75
Lab materials	PHP 3,000
Research Fee	PHP 12,000
TOTAL	**PHP 51,485.47**

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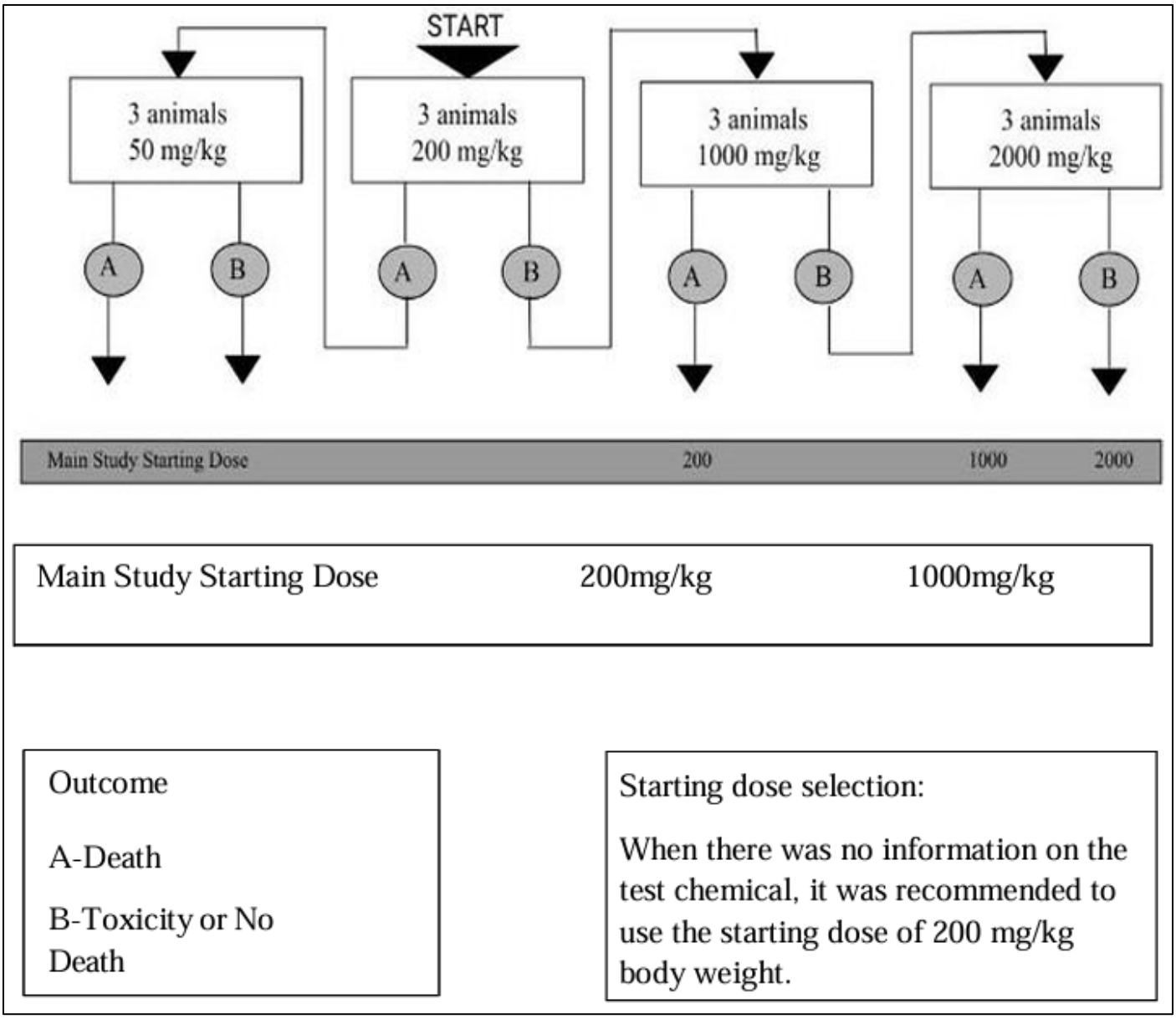
APPENDIX C
OECD 402:



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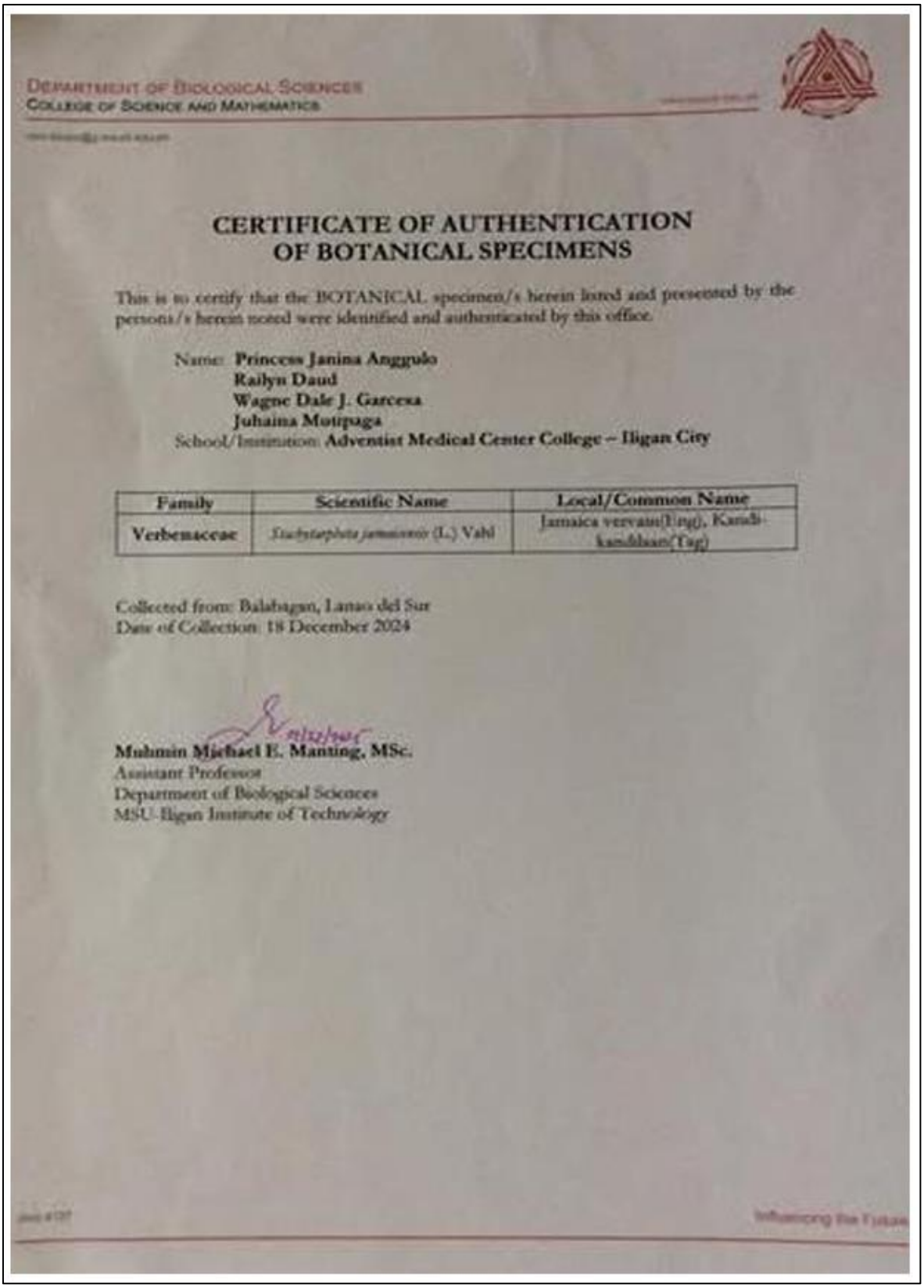
APPENDIX D
PLANT AUTHENTICATION CERTIFICATE



DEPARTMENT OF BIOLOGICAL SCIENCES
COLLEGE OF SCIENCE AND MATHEMATICS

CERTIFICATE OF AUTHENTICATION OF BOTANICAL SPECIMENS

This is to certify that the BOTANICAL specimen/s herein listed and presented by the person/s herein noted were identified and authenticated by this office.

Name: **Princess Janina Anggulo**
Railyn Daud
Wagne Dale J. Garcesa
Juhaina Mutpuga
School/Institution: **Adventist Medical Center College – Iligan City**

Family	Scientific Name	Local/Common Name
Verbenaceae	*Stachytarpheta jamaicensis* (L.) Vahl	Jamaica vervain(Engl), Kandikandilaan(Tag)

Collected from: Balabagan, Lanao del Sur
Date of Collection: 18 December 2024

Muhmin Michael E. Manting, MSc.
Assistant Professor
Department of Biological Sciences
MSU-Iligan Institute of Technology

Influencing the Future

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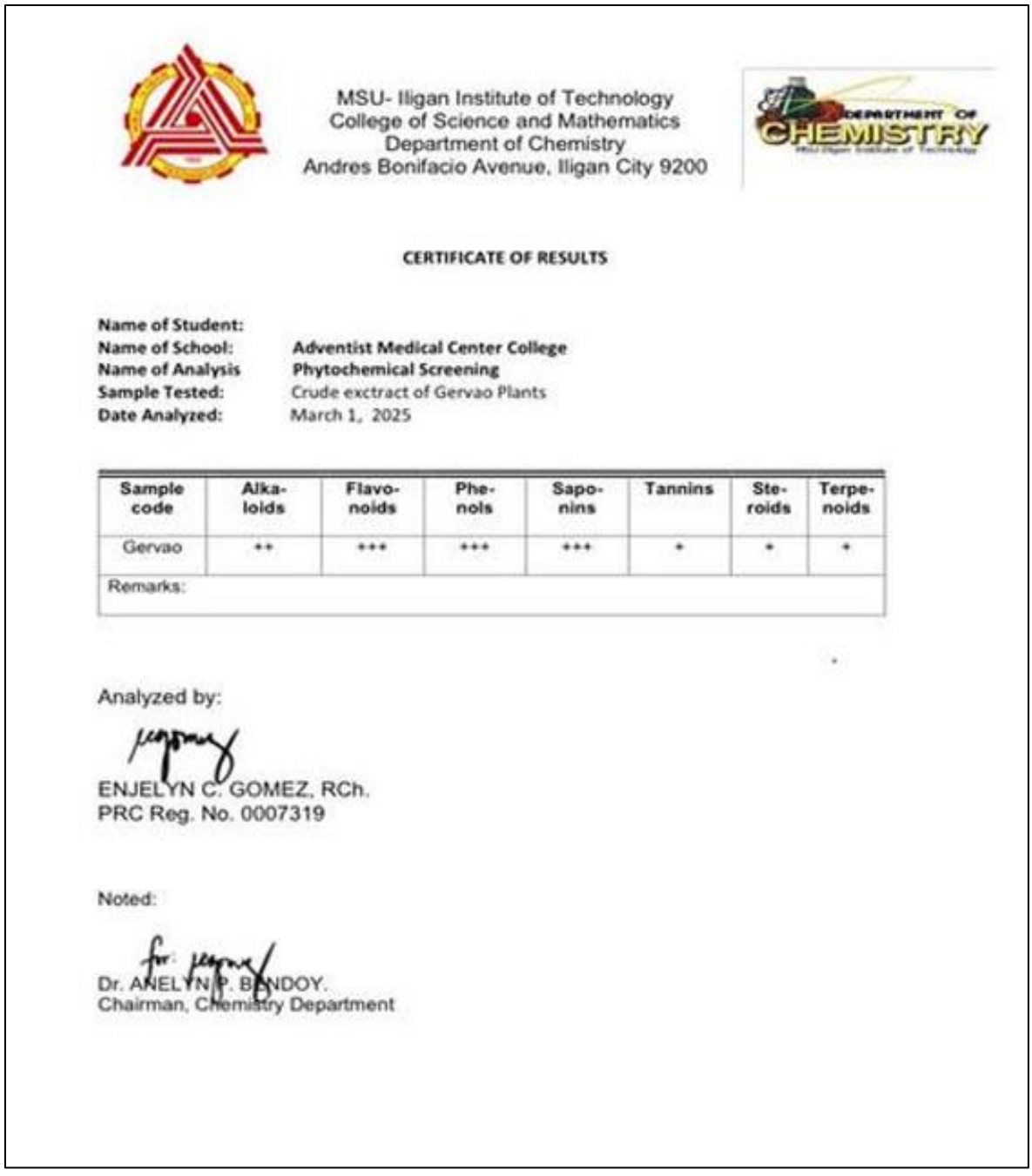
APPENDIX E
PHYTOCHEMICAL SCREENING



MSU- Iligan Institute of Technology
College of Science and Mathematics
Department of Chemistry
Andres Bonifacio Avenue, Iligan City 9200

DEPARTMENT OF CHEMISTRY

CERTIFICATE OF RESULTS

Name of Student:
Name of School: **Adventist Medical Center College**
Name of Analysis **Phytochemical Screening**
Sample Tested: Crude exctract of Gervao Plants
Date Analyzed: March 1, 2025

Sample code	Alka-loids	Flavo-noids	Phe-nols	Sapo-nins	Tannins	Ste-roids	Terpe-noids
Gervao	++	+++	+++	+++	+	+	+
Remarks:							

Analyzed by:

ENJELYN C. GOMEZ, RCh.
PRC Reg. No. 0007319

Noted:

for
Dr. ANELYN P. BENDOY.
Chairman, Chemistry Department

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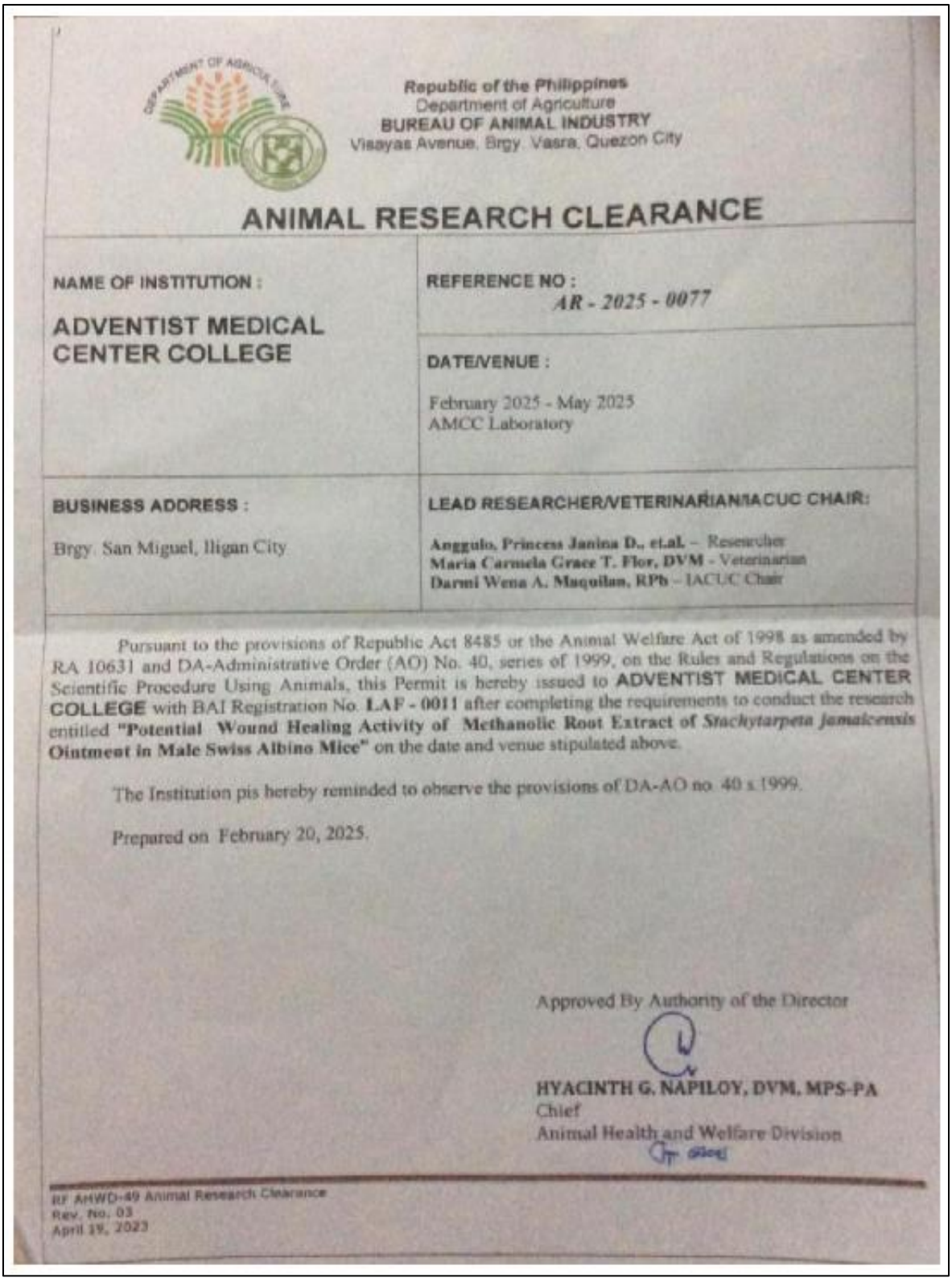
APPENDIX F
ANIMAL RESEARCH PERMIT



Republic of the Philippines
Department of Agriculture
BUREAU OF ANIMAL INDUSTRY
Visayas Avenue, Brgy. Vasra, Quezon City

ANIMAL RESEARCH CLEARANCE

NAME OF INSTITUTION : ADVENTIST MEDICAL CENTER COLLEGE	REFERENCE NO : *AR - 2025 - 0077* DATE/VENUE : February 2025 - May 2025 AMCC Laboratory
BUSINESS ADDRESS : Brgy. San Miguel, Iligan City	LEAD RESEARCHER/VETERINARIAN/IACUC CHAIR: **Anggulo, Princess Janina D., et.al.** – Researcher **Maria Carmela Grace T. Flor, DVM** - Veterinarian **Darmi Wena A. Maquilan, RPh** – IACUC Chair

Pursuant to the provisions of Republic Act 8485 or the Animal Welfare Act of 1998 as amended by RA 10631 and DA-Administrative Order (AO) No. 40, series of 1999, on the Rules and Regulations on the Scientific Procedure Using Animals, this Permit is hereby issued to **ADVENTIST MEDICAL CENTER COLLEGE** with BAI Registration No. **LAF - 0011** after completing the requirements to conduct the research entitled **"Potential Wound Healing Activity of Methanolic Root Extract of *Stachytarpeta jamaicensis* Ointment in Male Swiss Albino Mice"** on the date and venue stipulated above.

The Institution pis hereby reminded to observe the provisions of DA-AO no. 40 s.1999.

Prepared on February 20, 2025.

Approved By Authority of the Director

HYACINTH G. NAPILOY, DVM, MPS-PA
Chief
Animal Health and Welfare Division

RF AHWD-49 Animal Research Clearance
Rev. No. 03
April 19, 2023

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jul418

APPENDIX G
CERTIFICATE OF ANALYSIS (POVIDONE IODINE POWDER)

FOREVER

南京九佳科技有限公司

Nanjing Forever Pharmacy Co., Ltd.

地址：南京市栖霞区新港开发区27号

Certificate of Analysis

检 验 报 告 书

产品名称 Product Name	Pure Povidone Iodine Powder	CAS No.	25655-41-8
批号 Batch No	N43251898	数量 Quantity	100G
生产日期 Manufacture Date	2025.01.24	有效日期 Exp. Date	2028.01.23
检测日期 Test Date	2025.01.25	储存 Storage	RT

检测项目 Inspection Items	检测标准 Standard	检测结果 Results
APPEARANCE	YELLOW TO BROWN-RED POWDER	YELLOW POWDER
LOSS ON DRYING	≤8%	3.60%
AVAILABLE IODINE	9-12%	10.5%
PH	1.5-5	3.0
IODIDE ION	≤6.6%	2.7%
SULFATE ASH	≤0.025%	0.016%
HEAVY METAL (AS PB)	≤20PPM	< 20PPM
INFRARED SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS TO STRUCTURE

结论：按企业标准检验，符合公司标准

CONCLUSION: COMPLIED WITH COMPANY STANDARD

分析员(ANALYST) 审核(CHECKED) 质量经理(QC MANAGER)

质检专用章 Quality Inspection

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APPENDIX H
CERTIFICATE OF ANALYSIS (STEARIC ACID)

chem-supply

Product Specification

Product Name: **STEARIC ACID LR**

Alternate Name(s): Octadecanoic acid; n-octadecanoic acid; 1-heptadecanecarboxylic acid.

Description
White or yellowish-white flakes. Odour resembles fats and oils. It is a mixture of solid organic acids obtained from fats consisting chiefly of Stearic Acid and Palmitic Acid.

Properties
Chemical Formula:
Molecular Weight: 284.48

Product Code: SL049
CAS No. 57-11-4

General Information
Insoluble in water. Soluble in alcohol, ether, chloroform, carbon disulfide and carbon tetrachloride. Incompatible with strong oxidisers and strong bases. Combustible.

Hazard and Safety Data

UN Group:	None Allocated
Class:	None Allocated
UN Number:	None Allocated
Hazchem code:	None Allocated
CS MSDS Code:	1CH6Y
Poison schedule:	Not Scheduled
Emergency Procedure Guide No.:	N/A

Quality Specification
Assay: 48 - 56% (as C18)

Specific Properties and Impurities (Typical levels)

Acid value	206 - 211
Saponification value	207 - 212
Iodine value	≤ 0.5
Titre, °C	55 - 56
Colour, Lovibond 5.25"	≤ 0.3 Red, ≤ 3.0 Yellow
Fatty acid composition, %	
C12	< 1
C14	< 2
C16	43 - 50
C18	48 - 56
C18-1	Trace
C20	< 1

Rev. No: 3

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APPENDIX I
CERTIFICATE OF ANALYSIS (GLYCERYL STEARATE)

FOREVER

南京九佳科技有限公司
Nanjing Forever Pharmacy Co., Ltd.
地址：南京市栖霞区新港开发区27号

Certificate of Analysis
检 验 报 告 书

产品名称 Product Name	Glyceryl Stearate	CAS No.	11099-07-3
批号 Batch No	N15690204	数量 Quantity	5G
生产日期 Manufacture Date	2025.01.26	有效日期 Exp. Date	2028.01.25
检测日期 Test Date	2025.01.27	储存 Storage	2-8°C

检测项目 Inspection Items	检测标准 Standard	检测结果 Results
APPEARANCE	MILKY WHITE POWDER SOLID	MILKY WHITE POWDER SOLID
PURITY	≥98%	98.37%
WATER BY KARL FISCHER	≤1%	0.31%
SAPONIFICATION VALUE	150-165 MGKOH/G	160 MGKOH/G
ACID VALUE	≤5 MGKOH/G	3 MGKOH/G
NMR	CONFORMS TO STRUCTURE	CONFORMS TO STRUCTURE
INFRARED SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS TO STRUCTURE

结论：按企业标准检验，符合公司标准
CONCLUSION: COMPLIED WITH COMPANY STANDARD

分析员(ANALYST) 审核(CHECKED) 质量经理(QC MANAGER)

质检专用章 Quality Inspection

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APPENDIX J
CERTIFICATE ANALYSIS OF (GLYCERIN)

chem-supply

Product Specification

Product Name: **GLYCEROL LR**

Alternate Name(s) Glycerine; 1,2,3-propanetriol; trihydroxypropane; glycerin; glycyl alcohol.

Description
Clear colourless syrupy liquid; odourless; sweet taste; hygroscopic.

Properties
Chemical Formula: $C_3H_8O_3$
Molecular Weight: 92.09

Product Code: GL010
CAS No. 56-81-5

General Information:
Soluble in alcohol and insoluble in ether, benzene and chloroform. Combustible, may explode upon contact with strong oxidizing agents. About 0.6 times as sweet as cane sugar.

Hazard and Safety Data

UN Group:	None Allocated
Class:	None Allocated
UN Number:	None Allocated
Hazchem code:	None Allocated
CS MSDS Code:	1CH30
Poison schedule:	Not Scheduled
Emergency Procedure Guide No.:	N/A

Quality Specification
Assay: 99.0% min.

Specific Properties and Impurities [Typical levels]:

Vapour Pressure	0.0025 mm @ 50 °C
Vapour Density	3.17
pH Value	Neutral to litmus
Solubility in Water	Soluble
Refractive Index	1.474
Relative Density	1.2625
Autoignition Temperature	392 °C
Boiling Point	290 °C
Flash Point	199 °C
Melting Point	18 °C
Halgenated compounds	≤ 0.0035%
Heavy Metals	≤ 0.0005%
Sulphated Ash	≤ 0.01%

Chem-Supply Pty Ltd - An ISO 9001:2008 Accredited Company
38 - 50 Bedford Street, Gillman SA 5013, Australia ABN 19 008 264 211 PO Box 201, Port Adelaide SA 5015, Australia
Telephone +61 8 8440 2000 Fax +61 8 8440 2001 E-mail: sales@chemsupply.com.au Web: www.chemsupply.com.au

Rev. No: 2

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jul418

APPENDIX K
CERTIFICATE ANALYSIS OF (PETROLEUM JELLY)

LOBA Chemie

CERTIFICATE OF ANALYSIS

Product Name : PETROLEUM JELLY WHITE Extra Pure

Mol. Formula	:	Mol. Weight	:
Code No.	: [illegible]	CAS No.	: [illegible]
Mfg. Date	: [illegible]	Exp. Date	: [illegible]
HAZ. / P.G.	:	UN No.	:

Sr.	Tests	Specifications	Results
1	Appearance	[illegible]	[illegible]
2	Melting point	[illegible]	[illegible]
3	[illegible]	[illegible]	[illegible]

[illegible] LOBA CHEMIE PVT. LTD.

LOBA CHEMIE PVT. LTD.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jul418

APPENDIX L
CERTIFICATE ANALYSIS OF (CETYL ALCOHOL)

chem-supply

Product Specification

Product Name: CETYL ALCOHOL LR

Alternate Name(s): Cetylic alcohol; 1-Hexadecanol; Palmityl alcohol; Primary hexadecyl alcohol.

Description
White waxy solid; odourless.

Properties
Chemical Formula: $C_{16}H_{34}O$
Molecular Weight: 242.43

Product Code: CL044
CAS No. 36653-82-4

General Information:
Soluble in diethyl ether, acetone, chloroform; slightly soluble in alcohol. Insoluble in water. Incompatible with oxidising agents and strong acids.

Quality Specification
Assay: 90.0 % min.

Specific Properties and Impurities [Typical levels]:

Acid value (mgKOH/g)	≤ 0.5
Colour (Hazen)	≤ 40
Iodine value (mgKOH/g)	≤ 0.5
Water content	≤ 0.25 %
Ester value	≤ 0.5

Hazard and Safety Data

UN Group:	None Allocated
Class:	None Allocated
UN Number:	None Allocated
Hazchem code:	None Allocated
CS MSDS Code:	1CH1T
Poison schedule:	Not Scheduled
Emergency Procedure Guide No.:	N/A

Chem-Supply Pty Ltd - An ISO 9001:2000 Accredited Company
38 - 50 Bedford Street, Gillman SA 5013, Australia ABN 19 008 264 211 PO Box 201, Port Adelaide SA 5015, Australia
Telephone +61 8 8440 2000 Fax +61 8 8440 2001 E-mail: sales@chemsupply.com.au Web: www.chemsupply.com.au

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APPENDIX M
DOCUMENTATIONS

Collecting *S. jamaicensis* root

Washing the collected root in running water

Air-drying of collected *S. jamaicensis* roots.

After 2 to 3 months the roots are fully dried

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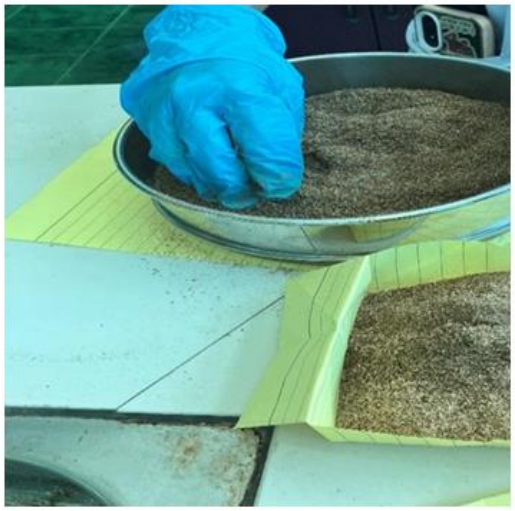
Cutting of dried roots

Powdering of *S. jamaicensis* root

Sieving of dried roots using sieve 60



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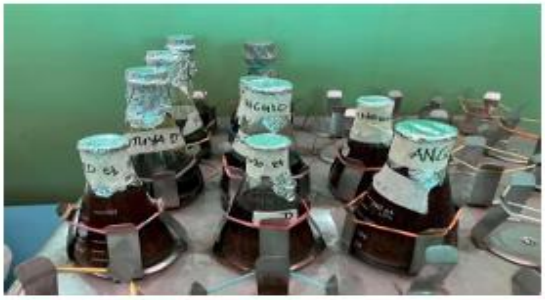
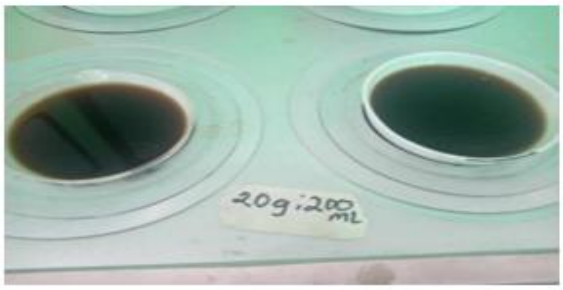
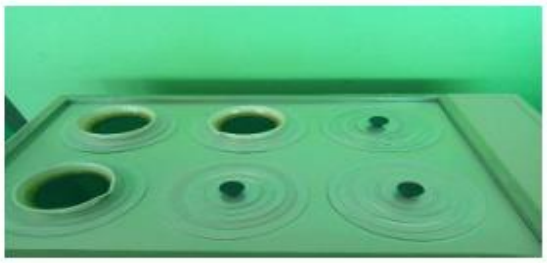
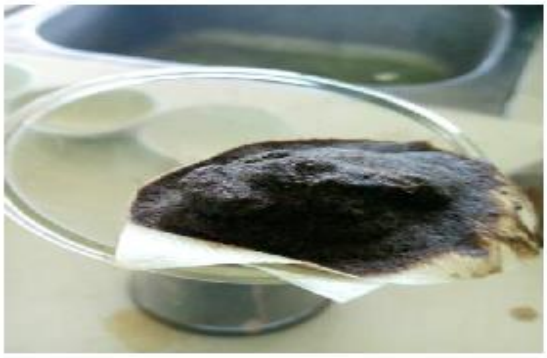
Macerating the methanolic solution for extract for 72 hours using maceration







Filtering the macerated methanolic root extract



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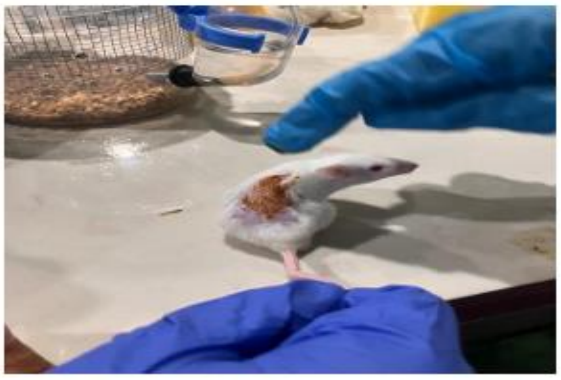
Application of OECD (50mg/kg,200mg/kg,1000mg/kg,2000mg/kg)


Preparation of Positive Control Ointment





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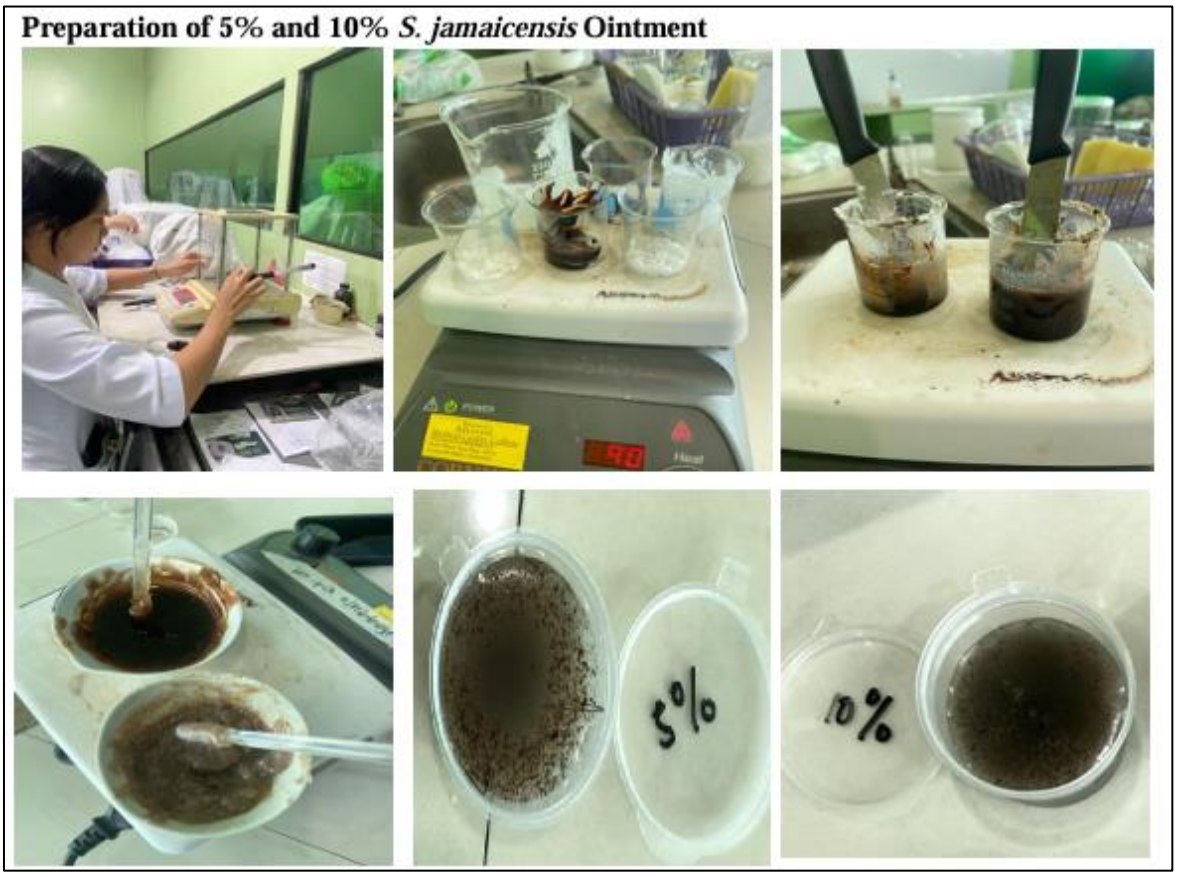
Preparation of Negative Control Ointment

Preparation of 5% and 10% *S. jamaicensis* Ointment



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Performing Excision Wound Model

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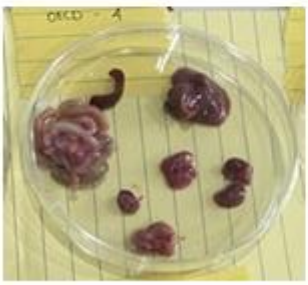
Mice Organs in OECD 402 Acute Dermal Toxicity Test

Mice Id	Organs
M1 (50mg/kg)	
M2 (50mg/kg)	
M3 (50mg/kg)	
M1 (200mg/kg)	

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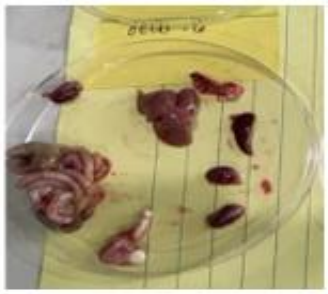
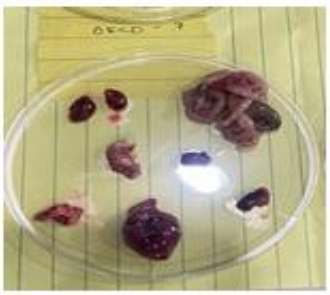
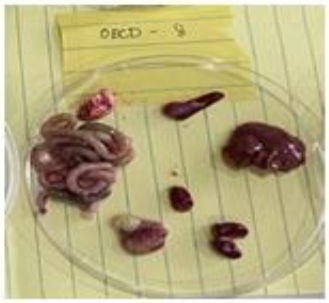
M2 (200mg/kg)	
M3 (200mg/kg)	
M1 (1,000mg/kg)	
M2 (1,000mg/kg)	

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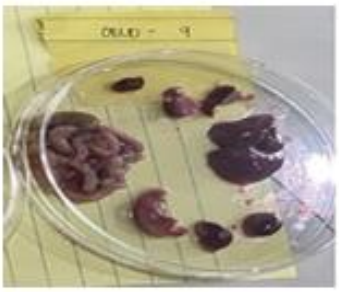
M3 (1,000mg/kg)	
M1 (2,000mg/kg)	
M2 (2,000mg/kg)	
M3 (2,000mg/kg)	

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APPENDIX N
RAW DATA

Mouse 1	write yes or no:	30 minutes	1 hour	2 hours	4 hours	6 hours	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM
Skin:	Redness	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Blisters	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Scratching	no	no	no	no	no	no	no	no	no	no		no		no		no		no
	fur loss	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Mucous Membranes:	Eyes red or draining	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	drooling	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Breathing patterns:	gaping mouth	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	panting	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Nervous system:	agitation	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	tremors	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	seizures	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	oversensitive to touch	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Mouse 2	write yes or no:																		
Skin:	Redness	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Blisters	no		no	no	no	no	no	no	no	no								
	Scratching	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	fur loss	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Mucous Membranes:	Eyes red or draining	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	drooling	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Breathing patterns:	gaping mouth	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	panting	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Nervous system:	agitation	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	tremors	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	seizures	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	oversensitive to touch	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no

MOUSE 3	write yes or no:		no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Skin:	Redness	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Blisters	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Scratching	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	fur loss	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Mucous Membranes:	Eyes red or draining	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	drooling	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Breathing patterns:	gaping mouth	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	panting		no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Nervous system:	agitation	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	tremors	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	seizures	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	oversensitive to touch	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no

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Second Batch ... Trial with 200mg/kg dose:		DAY 0: record time of application: _____					DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14
Mouse 1	write yes or no:	30 minutes	1 hour	2 hours	4 hours	6 hours	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM		
Skin:	Redness	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Blisters	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Scratching	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	fur loss	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Mucous Membranes:	Eyes red or draining	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	drooling	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Breathing patterns:	gaping mouth																		
	panting	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Nervous system:	agitation	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	tremors	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	seizures	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	oversensitive to touch	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no

Mouse 2	write yes or no:	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Skin:	Redness	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Blisters	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Scratching	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	fur loss	no																	
Mucous Membranes:	Eyes red or draining																		
	drooling	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Breathing patterns:	gaping mouth	no		no		no		no		no			no		no		no		no
	panting	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Nervous system:	agitation	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	tremors	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	seizures	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	oversensitive to touch	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Mouse 3	write yes or no:	no		no		no		no		no			no		no		no		no
Skin:	Redness	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Blisters	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Scratching	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	fur loss	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Mucous Membranes:	Eyes red or draining	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	drooling	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Breathing patterns:	gaping mouth	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	panting	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Nervous system:	agitation	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	tremors	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	seizures	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	oversensitive to touch	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no

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Mouse 2	write yes or no:	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Skin:	Redness	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Blisters	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Scratching	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	fur loss	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Mucous Membranes:	Eyes red or draining	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	drooling	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Breathing patterns:	gaping mouth	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	panting	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Nervous system:	agitation	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	tremors	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	seizures	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	oversensitive to touch	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Mouse 3	write yes or no:	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Skin:	Redness	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Blisters	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Scratching	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	fur loss	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Mucous Membranes:	Eyes red or draining	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	drooling	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Breathing patterns:	gaping mouth	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	panting	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Nervous system:	agitation	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	tremors	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	seizures	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	oversensitive to touch	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no

Second Batch ... Trial with 1000/kg dose:		DAY 0: record time of application: _____					DAY2	DAY3	DAY4	DAY5	DAY6	DAY7	DAY8	DAY9	DAY10	DAY11	DAY12	DAY13	DAY14
Mouse 1	write yes or no:	30 minutes	1 hour	2 hours	4 hours	6 hours	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM		
Skin:	Redness	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Blisters	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Scratching	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	fur loss	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Mucous Membranes:	Eyes red or draining	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	drooling	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Breathing patterns:	gaping mouth	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	panting	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Nervous system:	agitation	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	tremors	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	seizures	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	oversensitive to touch	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no

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Second Batch ... Trial with 2000/kg dose:		DAY 0: record time of application: _____					DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14
Mouse 1	write yes or no:	30 minutes	1 hour	2 hours	4 hours	6 hours	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM	8AM	no	no	no
Skin:	Redness	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Blisters	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Scratching	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	fur loss	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Mucous Membranes:	Eyes red or draining	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	drooling	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Breathing patterns:	gaping mouth	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	panting	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Nervous system:	agitation	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	tremors	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	seizures	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	oversensitive to touch	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no

Mouse 2	write yes or no:	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Skin:	Redness	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Blisters	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Scratching	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	fur loss	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Mucous Membranes:	Eyes red or draining	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	drooling	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Breathing patterns:	gaping mouth	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	panting	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Nervous system:	agitation	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	tremors	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	seizures	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	oversensitive to touch	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Mouse 3	write yes or no:	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Skin:	Redness	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Blisters	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	Scratching	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	fur loss	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Mucous Membranes:	Eyes red or draining	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	drooling	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Breathing patterns:	gaping mouth	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	panting	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Nervous system:	agitation	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	tremors	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	seizures	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
	oversensitive to touch	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no

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Necropsy Results of Acute Dermal Toxicity Test (OECD 402)

Mouse ID	Liver (Weight in g)	Spleen (Weight in g)	Right Kidney (Weight in g)	Left Kidney (Weight in g)	Lungs (Weight in g)
M1(50mg/kg) 23.7g	3.837g	0.112g	0.203g	0.242g	0.437g
M2(50mg/kg 26.2g)	1.800g	0.156g	0.245g	0.274g	0.271g
M3(50mg/kg) 25.9g	0.627g	0.124g	0.122g	0.125g	0.301g
M1(200mg/kg) 25.8g	1.795g	0.206g	0.151g	1.149g	0.433g
M2(200mg/kg) 24.5g	1.613g	0.110g	0.154g	0.155g	0.183g
M3(200mg/kg) 22.5g	1.410g	0.203g	0.160g	0.162g	0.389g
M1(1,000mg/kg) 21.9g	1.397g	0.149g	0.170g	0.172g	0.321g
M2(1,000mg/kg) 22.0g	0.368g	0.206g	0.154g	0.155g	0.490g
M3(1,000mg/kg) 25.3g	1.415g	0.190g	0.134g	0.136g	0.225g
M1(2,000mg/kg) 24.4g	1.470g	0.220g	0.231g	0.235g	0.177g
M2(2,000mg/kg) 27.8g	1.589g	0.216g	0.200g	0.205g	0.205g
M3(2,000mg/kg) 28.9g	1.949g	0.345g	0.321g	0.221g	0.305g

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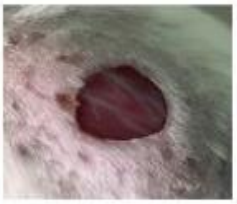
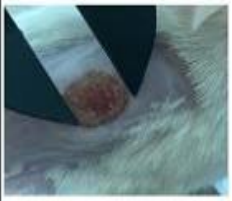
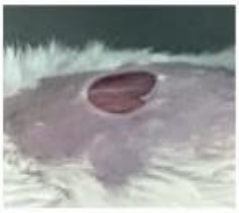
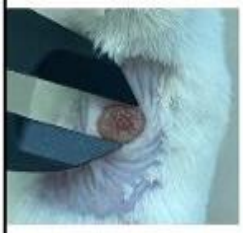
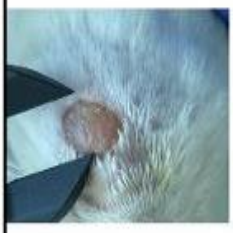
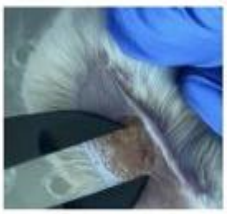
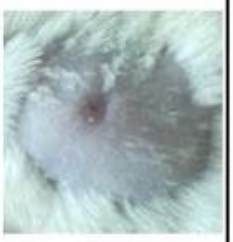
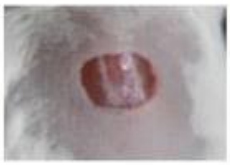
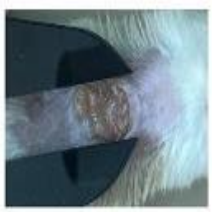
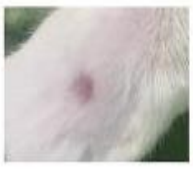
Positive Control

Mouse ID	Day 0	Day 3	Day 7	Day 14
M1				
M2				
M3				
M4				
M5				

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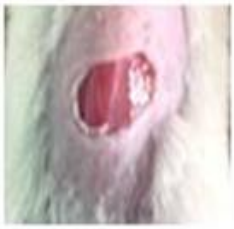
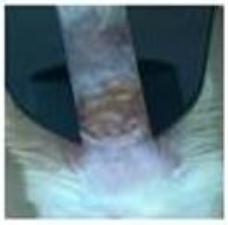
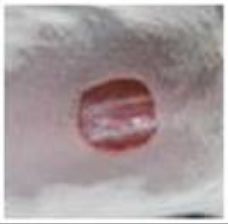
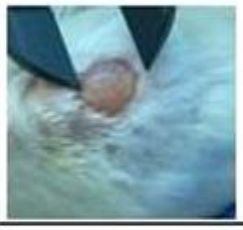
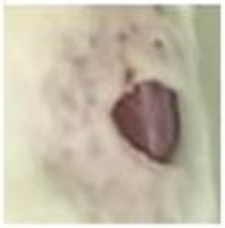
Negative Control

Mouse ID	Day 0	Day 3	Day 7	Day 14
M1				
M2				
M3				
M4				
M5				

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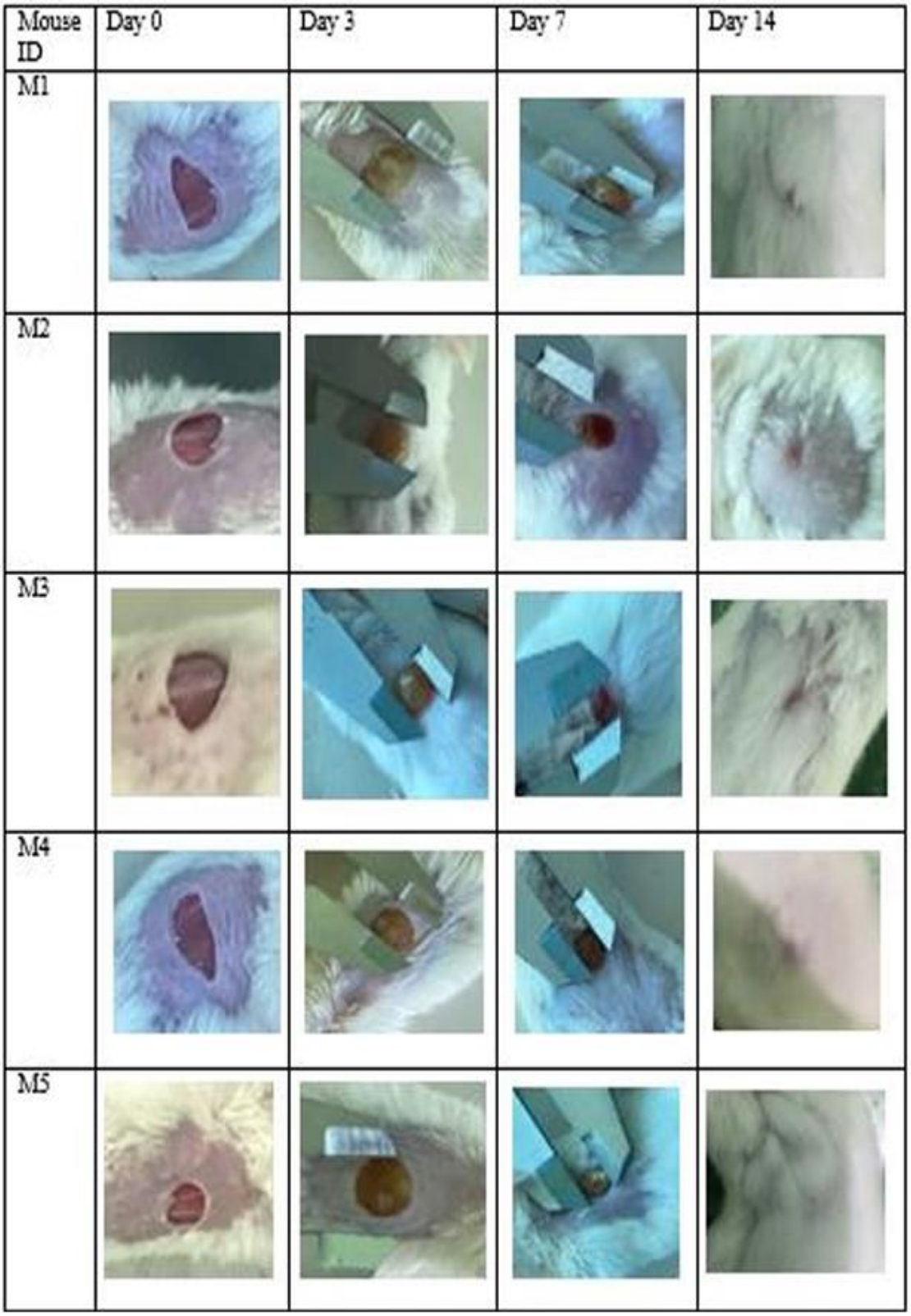
5% of *S. jamaicensis* Ointment



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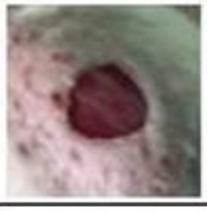
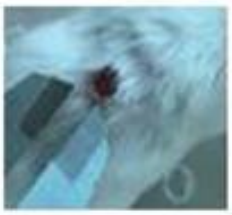
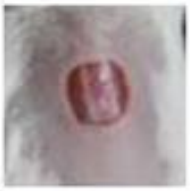
10% of *S. jamaicensis* Ointment

Mouse ID	Day 0	Day 3	Day 7	Day 14
M1				
M2				
M3				
M4				
M5				

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Raw Data from day 0- day 14

	Positive control	Negative control	5% concentration	10% concentration
DAY 0	50.24	50.24	50.24	50.24
DAY 1	50.24	50.24	50.24	50.24
DAY 2	45.47	49.24	42.53	36.62
DAY 3	32.47	48.50	33.06	25.78
DAY 4	24.45	45.95	23.32	14.93
DAY 5	16.40	43.12	15.13	9.47
DAY 6	12.76	33.27	9.51	4.31
DAY 7	7.39	31.10	5.16	1.84
DAY 8	4.82	25.62	2.13	0.06
DAY 9	4.28	23.76	0.10	0.02
DAY 10	0.90	17.79	0.02	0.00
DAY 11	0.64	8.98	0.00	0.00
DAY 12	0.06	5.74	0.00	0.00
DAY 13	0.03	5.48	0.00	0.00
DAY 14	0.00	1.63	0.00	0.00