



ARUGABON: A FORMULATED ORGANIC OPEN WOUND-HEALING OINTMENT USING SAMBONG (*Blumea balsamifera*) LEAF EXTRACTS WITH GUAVA (*Psidium guajava*) SCENT

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Abstract: This study evaluated the efficacy of a formulated organic open wound-healing ointment using Sambong (*Blumea balsamifera*) leaf extract with Guava (*Psidium guajava*) scent. The organic formulation, comprising plant-based extracts and oils, demonstrated notable therapeutic effects. At a 50% concentration, Sambong extract significantly reduced wound size by 94.48%, slightly lower than Betadine ointment's 97.76% reduction. However, Sambong-treated wounds exhibited higher tensile strength (73.39 g/mm²) compared to Betadine (13.51 g/mm²) and distilled water (10.33 g/mm²), suggesting stronger, more resilient healed tissue. Sambong also showed partial antibacterial activity against *Staphylococcus aureus*, with a zone of inhibition between 9 and 10 mm, contributing to wound healing by potentially reducing bacterial colonization. The ointment's pH (5.33) aligns with the natural skin pH, promoting an optimal environment for healing without disrupting the skin barrier. Additionally, patch tests indicated no adverse reactions, highlighting its safety for skin use. Statistical analysis confirmed significant differences in wound reduction, antibacterial efficacy, and body weight changes among groups, with the Sambong group showing a slight weight reduction. While Betadine was slightly more effective in reducing wound area, the higher tensile strength of Sambong-treated wounds suggests better wound integrity, which is beneficial for complex or deeper wounds. In sum, this Sambong-based ointment shows promise as a natural alternative or complement to conventional wound treatments, with further research recommended to enhance its antibacterial properties and examine histopathological healing characteristics.

INTRODUCTION

People sometimes have little mishaps in daily life that cause acute injuries or open wounds. Although these injuries seem minor, poor care can cause major medical problems, including delayed wound healing and infections. Any traumatic damage that compromises the integrity of the skin and exposes underlying tissues to outside surroundings and possible microorganisms is known as an open wound. Most open wounds are regarded as light; thus, they are usually treated at home with self-care practices (Rodrick, 2018). On the other hand, incorrect treatment could raise the chance of bacterial infection, affecting health and recovery greatly.

Ensuring good healing while preventing infections is one of the main difficulties in wound treatment. Many people use over-the-counter (OTC) ointments and antiseptics to heal open wounds, but growing interest in organic and plant-based substitutes results from questions about chemical-based therapies. Long used in traditional medicine for wound healing, medicinal plants highlight the need for safe and scientifically confirmed herbal therapies. In response to this gap, this work focused on developing an organic open-wound healing ointment using Sambong (*Blumea balsamifera*) leaf crude extractions, infused with Guava (*Psidium guajava*) smell, as a natural alternative to standard wound care products.

Furthermore, guaranteeing appropriate tissue regeneration and avoiding infections depends on effective wound treatment. The growing desire for plant-based, organic medicines emphasizes the need for scientific investigation of herbal cures. This research sought to offer a safer, more easily available, and environmentally friendly alternative to synthetic wound care products, helping to shape a natural one. Moreover, the research fits the global trend toward natural and traditional medicine, which remains the main healthcare method used by millions of people all over (Shedoeva, Leavesley, Upton, & Fan, 2019). This study encourages the merging of indigenous wisdom with modern scientific techniques by developing an organic ointment, therefore possibly aiding people who favor holistic therapeutic approaches.

Additionally, this study was aligned with the Traditional and Alternative Medicine Act (TAMA) of 1997 (Republic Act No. 8423) since it guarantees the safety, efficacy, and accessibility of herbal and alternative medicines through research and development, ensuring their relevance. TAMA encourages the integration of traditional remedies into contemporary healthcare and

offers the legal basis for researching medicinal plants since the study aimed to develop an organic open-wound healing salve utilizing Sambong (*Blumea balsamifera*) leaf crude extracts. Besides, this study supports the Philippine Institute of Traditional and Alternative Health Care (PITAHC) in its endeavor to promote natural healing techniques by matching with TAMA, therefore helping to provide scientific confirmation of herbal medicine. The law validates the efforts of the research to investigate plant-based wound treatment, therefore enabling government acceptance, control, and extensive application of the developed ointment in the Philippines.

Indeed, according to Pang, Wang, Fan, Chen, Yu, Hu, Wang, & Yuan, (2014), plants have been extensively applied in the pharmaceutical sector for ointment manufacture, particularly for treating open wounds. For millennia, traditional medicine has benefited much from medicinal herbs having antibacterial and anti-inflammatory effects. Many studies have shown how well plant-based chemicals promote wound healing, therefore supporting their possible viability as a substitute for synthetic formulations (Traversaz, Umehara, Watanabe, Rachidi, Sève, & Souard, 2023). Particularly, Sambong has been identified for its medicinal qualities, including wound-healing, antibacterial, and antioxidant action (Pang et al., 2014).

In light of these claims and studies on the healing properties of certain medicinal plants in the field of natural remedies and holistic health, the researchers were motivated to develop an organic open wound-healing ointment that would utilize Sambong (*Blumea balsamifera*) leaf crude extracts with a Guava (*Psidium guajava*) scent. The synergistic properties of Sambong and Guava were thought to offer a viable remedy for open-wound healing. These components have been utilized as medicinal plants throughout Southeast Asia, including the Philippines, for an extended period and are esteemed for their various therapeutic characteristics. While Guava leaf extract is widely recognized for its antibacterial properties, Sambong is an excellent natural alternative to conventional wound care remedies due to its wound-healing, antimicrobial, antioxidant, and anti-inflammatory properties (Pang et al., 2014). Combining Sambong's healing properties with Guava's strong antioxidant and antibacterial benefits to create a mild, effective cure based on traditional herbal knowledge, this study blended these two plants into an organic ointment meant to improve skin recovery and wound healing.

Also, in vitro and in vivo testing were crucial steps in formulating organic open-wound healing ointments for several important reasons, including their efficacy assessment, which assessed the potential healing properties of the formulated organic ointment at the cellular and molecular levels, safety evaluation, optimal evaluation, understanding mechanisms of action, and regulatory compliance, and real-world validation, which bridged the gap between laboratory experiments and clinical application, demonstrating how the ointment performs in real clinical settings.

In sum, in vitro and in vivo testing were indispensable in formulating organic open-wound healing ointments. They ensured that the ointment was not only effective but also safe, well-formulated, and capable of addressing the complex biological processes involved in wound healing. These tests were critical for both scientific validation and regulatory approval, ultimately leading to the formulation of reliable and trustworthy organic wound care solutions.

Objectives of the Study

This study formulated an organic open wound-healing ointment using the Sambong (*Blumea balsamifera*) leaf extracts with Guava (*Psidium guajava*) scent.

Specifically, it performed the following tasks:

1. Formulate an open wound-healing ointment utilizing the adopted but modified protocol of Fuentes et al. (2023) using Sambong (*Blumea balsamifera*) leaf extracts with Guava (*Psidium guajava*) scent.
2. Test the properties of the formulated organic open wound-healing ointment in terms of its:
 - 2.1 Antibacterial Properties;
 - 2.2 pH Level;
 - 2.3 Irritation;
 - 2.4 Wound-Healing Properties; and
 - 2.5 Tensile Strength.
3. Compare the significant difference between the treatments and the controls regarding their antibacterial properties, the mean body weight of sample mice, size of wounds, and tensile strength.

Research Questions

Considering the objectives of the study, the researchers sought answers to the following questions:

1. How can an open wound-healing ointment utilizing the adopted but modified protocol of Fuentes et al. (2023) using Sambong (*Blumea balsamifera*) leaf extracts with Guava (*Psidium guajava*) scent be formulated?
2. What are the results of the tests regarding the properties of the formulated organic open wound-healing ointment in terms of its:
 - 2.1 Antibacterial Properties;
 - 2.2 pH Level;
 - 2.3 Irritation;
 - 2.4 Wound-Healing Properties; and
 - 2.5 Tensile Strength?
3. Is there a significant difference between the treatments and the controls regarding their antibacterial properties, the mean body weight of sample mice, size of wounds, and tensile strength?

Hypothesis of the Study

H₀ There is no significant difference between the treatments and the controls regarding their antibacterial properties, the mean body weight of sample mice, the size of wounds, and tensile strength.

Significance of the Study

This study is hoped to be beneficial to the following:

Environmental and Botanical Sciences may benefit from formulating organic open-wound healing ointments in several ways, such as **Reduced Environmental Impact**. Organic ointments typically use natural ingredients and avoid synthetic chemicals that can have adverse environmental effects when produced, used, or disposed of. These ointments reduce pollution and minimize the release of harmful substances into the environment by opting for organic ingredients. These organic ingredients also tend to have a lower risk of causing potential harm to aquatic life, wildlife, and ecosystems.

Sustainable Sourcing: Many organic ointments prioritize using sustainably sourced plant-based ingredients like Sambong. This promotes responsible harvesting practices that help protect biodiversity and ecosystems. Sustainable sourcing can also support local communities that rely on these natural resources. Also, **Preservation of Medicinal Plants**: Using traditional medicinal plants like Sambong in organic ointments encourages the conservation of these valuable plant species. By recognizing the healing properties of these plants and integrating them into healthcare products, there is a greater incentive to protect and preserve their natural habitats.

Further, **Public Awareness and Education**: The formulation and promotion of organic wound healing products raise awareness about the environmental benefits of using natural, sustainable, and organic materials. It encourages consumers to make more eco-conscious choices in healthcare and skincare products.

Policy Implications also may benefit from this study because introducing organic wound healing products may prompt policymakers to establish or refine regulatory frameworks specific to such products. This can include standards for organic certification, labeling requirements, and safety assessments tailored to natural and organic ingredients. Also, policymakers may work to establish apparent safety and efficacy standards for organic wound healing ointments. These standards can help ensure that these products are safe and effective for consumers. Likewise, policymakers may develop guidelines for labeling and marketing organic ointments, including requirements for accurate ingredient listing, claims substantiation, and eco-friendly packaging. This helps consumers make informed choices and prevents deceptive marketing practices.

Moreover, this present study contributes to the policy regarding improving the rules and protocols followed by the combined plants in the form of ointment and to the processing of the products, ensuring the cleanliness of the substance used. Considering the existence of other studies that discuss the same aims as this study, the researchers took an oath to adhere to the rules that only measure the safety and reliability of the desired output of their product. Besides, the researchers desire to convey new knowledge for further researchers' references and to be able to contribute to the betterment of the treatment industry, specifically focusing on the effectiveness of combined Sambong (*Blumea balsamifera*) leaf extracts with Guava (*Psidium guajava*) scent, and their compatibility with each other and suitability as well as their diverse sensitivity to a variety of skins through testing.

The **Community** may benefit from this study because the availability of organic wound healing products may raise awareness about natural and eco-friendly healthcare options within the community. This may lead to a broader understanding of the benefits of organic products and a shift toward more sustainable healthcare choices. Traditional medicinal plants like Sambong have been used for generations. Formulating organic ointments incorporating these plants may help preserve and promote traditional healing practices, strengthening cultural ties and knowledge. By fostering sustainable practices and promoting local resources, the formulation of organic ointments contributes to community resilience. Communities become less dependent on external sources for healthcare solutions, which can be particularly important in remote or underserved areas.

The **STEM Students** may benefit from this study by engaging in research projects on formulating these ointments and gaining valuable hands-on experience in laboratory settings. They may learn about the formulation, testing, and optimization of organic wound healing products, which can be applied to various scientific disciplines. Also, developing organic open-wound healing ointments often involves overcoming complex scientific and technical challenges. STEM students may hone their problem-solving and critical thinking skills while working on innovative solutions to enhance the efficacy and safety of these products.

Furthermore, **STEM students** may take pride in contributing to the field of healthcare by formulating organic ointments that are safe, effective, and environmentally friendly. Their work may have a positive impact on patient outcomes and well-being. Students may learn about the properties and potential benefits of natural ingredients like Sambong. This knowledge may be applied to future research projects or careers such as natural product chemistry or herbal medicine.

Additionally, they may develop a greater awareness of the environmental implications of healthcare products. They may explore ways to reduce the ecological footprint of pharmaceuticals and contribute to sustainable healthcare practices. Similarly, successful research projects may allow STEM students to publish their findings in scientific journals or present their work at conferences. This may enhance their academic and professional profiles. Indeed, the knowledge and skills gained through involvement in formulating organic ointments may open up diverse career pathways in pharmaceuticals, healthcare, research and development, regulatory affairs, and environmental science, among others.

Future Researchers may benefit from this study because the formulation of organic open-wound healing ointments creates a dynamic and evolving research landscape for future researchers. It offers opportunities for innovation, interdisciplinary collaboration, and contributions to sustainable healthcare practices. As these ointments gain recognition and acceptance, they may provide a promising avenue for research that may benefit both patients and the environment. Furthermore, the recommendations presented in this study may serve as a guide for future researchers to explore other product formulation areas apart from the study materials, which may be reflected in the research gap.

Scope and Delimitation of the Study

This work was aimed at developing an organic open-wound healing ointment with Guava (*Psidium guajava*) aroma from Sambong (*Blumea balsamifera*) leaf crude extracts. The main goals of the study were to evaluate the tensile strength, pH level, irritation, wound-healing effectiveness, and antibacterial qualities of the produced ointment. It also found notable variations in terms of antibacterial activity, irritation, changes in body weight of sample mice, wound size reduction, and tensile strength between

treatments and controls. The study looked at herbal-based wound treatment, especially the healing qualities of Sambong leaf crude extracts mixed with Guava smell as an organic ointment basis. Under the field of alternative medicine and Pharmacological Sciences, the study sought to add to the mounting body of herbal medicinal research by combining scientific, experimental, and pharmacological viewpoints to investigate the efficacy of a naturally derived ointment relative to current synthetic substitutes.

Methodologically, the study used *in vivo* and *in vitro* experimental setups. Along with a pH test to ascertain the ointment's appropriateness for skin application, the *in vitro* phase included an antibacterial examination utilizing the Disk Diffusion technique against *Staphylococcus aureus*. The *in vivo* phase comprised wound-healing trials on Sprague Dawley mice (*Rattus norvegicus*) and testing of wound-healing efficiency carried out on human subjects under dermatological irritation assessments. Tensile strength tests were also done to see if the ointment may improve skin recovery. Under informed agreement from their guardians, the study comprised a subset of Grade 12 STEM students from Colon National High School for the patch test. Male Sprague Dawley mice were utilized under ethical standards in the wound-healing tests.

Moreover, the study took place at Colon National High School in Maasim, Sarangani Province, and laboratory studies were done at MSU-Gensan's Nutraceutical Laboratory. The gathered materials were from Purok Kamotes, Brgy. Kabatiol, Maasim, Sarangani Province; Purok River Site, and Brgy. Kablacan. Following a planned schedule spanning November 2023 to May 2024 for formulation, testing, and assessment, the research took place throughout the academic year 2023–2024.

Limitations of the Study

Though it covered a lot, the study had several limits. Focusing only on acute, superficial wounds, it neglected the long-term consequences of surgical, chronic, or deep-tissue injuries. Furthermore, lacking thorough phytochemical characterization, only Sambong leaf crude extracts were the main active component. Apart from other bacterial strains, the study focused on the antibacterial qualities of the ointment against *Staphylococcus aureus*. Furthermore, sophisticated spectroscopic techniques such as HPLC (High-Performance Liquid Chromatography) or FTIR (Fourier-Transform Infrared Spectroscopy) were not applied for chemical characterization. Without evaluating other commercial herbal ointments, the study's comparison consisted only of Povidone-iodine (Betadine) for wound-healing testing, Mupirocin for antibacterial testing as positive controls, and distilled water as a negative control.

Furthermore, the study eliminated those with sensitive skin problems, allergies, or pre-existing dermatological illnesses. To reduce variability in the wound-healing research, only male Sprague Dawley mice were employed. Moreover, present were certain methodological constraints that would have affected the consistency of the final product: variability in plant age, environmental conditions, and raw material composition. Also, influencing the generalizability of the results in both human and animal studies could be constraints in sample size.

Similarly, potential biases included observer bias since human mistakes could be introduced by hand measurements of tensile strength and wound size reduction. In addition, funding and time limits prevented a double-blind clinical trial from being carried out, and no pharmacokinetic or toxicological research was done to evaluate the active component's systemic absorption or metabolism.

RESEARCH METHODOLOGY

This study employed the quantitative method, particularly the true experimental design. Utilizing this design helped the researchers in formulating an organic open-wound healing ointment using Sambong (*Blumea balsamifera*) leaf extracts, and Guava (*Psidium guajava*) oil as its scent by adopting but modifying the formulation protocol from Fuentes et al. (2023), in testing the properties of the formulated organic open-wound healing ointment regarding antibacterial properties, pH level, irritation, wound-healing property, and tensile strength, and in comparing the significant difference between the treatments and the controls regarding antibacterial properties, irritation, the mean body weight of sample mice, size of wounds, and tensile strength in different concentrations applied.

According to Pubrica-Academy (2022), experimental research is a type of scientific examination in which one or more independent variables are changed and then applied to one or more dependent variables to see how they affect the latter. The effect of independent variables on dependent variables is frequently observed and recorded to help researchers reach a plausible conclusion about the link between these two variables. Further, experimental research designs, most commonly associated with laboratory test procedures, entail gathering quantitative data and doing statistical analysis during the study process.

Materials Used

Sambong (*Blumea balsamifera*) leaves served as the main organic material in making the ointment. Guava (*Psidium guajava*) essential oil served as the scent of the ointment; it is known for its sweet and slightly tart aroma, and guava is often described as having a scent reminiscent of apple, strawberry, and pine. Olive oil and coconut oil have a lot of vitamins, such as vitamins E and K, which are beneficial to the skin. Both are also good for skin moisturizing, and in this study, they served as the base for making an open wound-healing ointment.

Moreover, honey was used as one of the basic moisturizers that enhanced the efficacy of the ointment when applied to the skin, open wounds, and sunburns. Beeswax, which also helps heal skin conditions, served as an emulsifier of the formulated open wound-healing ointment. Distilled water was used as the final wash agent for Sambong leaves and was used to clean the tools before use. Ethanol (Ethyl Alcohol) was used in soaking the pulverized materials, i.e., dehydrated Sambong leaves, to generate the bioactive contents or metabolites in the materials and in disinfecting the materials and tools used during the laboratory process. Mupirocin served as the positive control of the researchers during their antibacterial test using the Disk Diffusion Method, while the Betadine served as the positive control during the researchers' *in vivo* testing. The anesthesia (Lidocaine) was used to anesthetize the mice during the incision. Skin plasters were used to cover the area where the samples of controls and treatments were applied for irritation tests, while tissue papers were used to dry up alcohol and other solvents.

pH strips were used to measure the acidity or alkalinity of the formulated ointment. Filter paper disks were used in conducting the disk diffusion method (Antibacterial test). The filter paper was used to filter the Sambong powder soaked in ethyl

alcohol to obtain the Sambong ethyl alcohol mixture. The cotton swab was used to handle filter paper disks and transfer them to a petri dish without introducing contamination.

Tools Used

A stirring rod was used to mix the ointment components thoroughly. Additionally, a Buchner funnel facilitated filtering to remove solid particles. An Erlenmeyer flask was used for precise measurement of liquid quantities, ensuring accuracy in the preparation process. A stainless spatula was employed for the accurate handling and transfer of materials. A glass jar was used as a container for the soaking of the powdered Sambong leaves. A Petri dish was used to culture bacteria. A beaker was used for holding, mixing, and heating liquids. Test tubes were used as containers in standardizing the bacteria (*Staphylococcus aureus*) using a Vortex mixer. The dropper facilitated the precise dispensing of liquids, allowing for controlled addition to experiments.

Allis forceps were used to hold the mice in place during the tensile strength test, while a Vernier caliper measured the size of the wounds during observation of in vivo tests and the size (in mm) of the zone of inhibition during the antimicrobial assay. An alcohol lamp was used to sterilize the mouth of the agar container; iris scissors were used for laceration in the mice, and a magnetic stirrer was used to mix solutions and ensure an even distribution of components. Weighing boats were used to hold and weigh solid and liquid ingredients accurately. A product container was used to store the Sambong ointment securely. Dehydrator trays were used to dry the Sambong leaves before processing. A glass funnel was used to transfer liquids neatly into containers without spills. Syringes were used for administering anesthesia to mice during the experiments.

Equipment Used

A Silver Crest blender and a NutriBullet blender were used to pulverize the dehydrated Sambong leaves. A laboratory dehydrator was used to decrease the moisture content of the Sambong leaves, making them crunchy and easy to crumble. An analytical balance was used to measure accurately the weight of the ingredients needed for the formulation. A top-loading balance was employed to measure the materials precisely. The consistency was brought to a syrup-like state using a water bath. The ethanol was extracted from the Sambong ethanolic extract using a rotary evaporator. To heat and melt the necessary components, including beeswax, a hot plate was utilized. A colony counter was used to read and identify the zone of inhibition (ZOI), while a vortex mixer was used to standardize the bacteria, *Staphylococcus aureus*. The Biobase Vertical Chemical Wet Bench was used for safely handling and applying the bacteria onto agar plates, featuring a chemical-resistant surface and a fume extraction system.

Paraphernalia Used

Laboratory gowns were worn to protect the wearer's clothing and skin from contamination and exposure to hazardous materials, chemicals, or biological agents. Face masks were used to prevent the inhalation of airborne contaminants and to protect against the spread of respiratory droplets that might carry infectious agents. Laboratory footwear was used to safeguard the feet from spills, splashes, and sharp objects, maintaining a clean and safe environment. Gloves were worn to protect the hands from direct contact with hazardous substances and to prevent cross-contamination between different areas or samples.

PROCEDURE

A. Preparation Phase

In the extraction process of the Sambong leaf extracts, a substantial quantity of leaves was gathered, ensuring their cleanliness before storing them in zip-lock bags. Within the laboratory setting, the leaves underwent a meticulous dehydration process overnight, carefully regulated at a temperature of 45°C. Following this initial step, the leaves were finely ground using blenders to facilitate further extraction. Subsequently, the ground leaves were immersed in a total of 9 liters of ethanolic alcohol, with varying volumes per batch, to initiate the extraction process. Upon completion of soaking, the mixture of Sambong and ethyl alcohol underwent a thorough filtration and rotary evaporation procedure, meticulously separating the ethanol from the plant's pure crude extracts. Following the separation process, the resulting pure extracts underwent a water-bathing process to achieve a desirable syrup-like consistency.

B. Formulation Phase

In formulating the base for the ointment in this study, the researcher adopted but modified the formulation protocol of Fuentes, Zulueta, Ave, Banes, Cabasag, Diana, Esperanza, Gustame, Maghanoy, and Panuncial (2023). Beeswax (24g), Olive Oil (48.12g), Coconut Oil (48.18g), and Honey (10.98g) were combined. The ointments formulated have different concentrations with the same amount of Guava scent (25 drops): 25%, 50%, 75%, and 100% concentrations. However, in mass production, the drops of Guava scent were increased to 30 drops as suggested by panelists to enhance the scent of the ointment.

C. In Vitro Test

Testing the Antibacterial Properties of the Formulated Open Wound-Healing Ointment

In vitro tests were conducted before advancing to more resource-intensive in vivo studies and clinical trials to assess efficacy, safety, and potential value. To identify which concentration has the greatest antibacterial properties, each of them went through the agar disc diffusion method. After incubating at 35–37 degrees Celsius for 48 hours, the agar plates were examined for microbial colonies. Finally, the Zone of Inhibition (ZOI) around the disks was measured and compared to standardized breakpoints to determine which among the concentrations was categorized as Inactive (0-9mm), Partially Active (10-13mm), Active (14-19mm), or Very Active (>19mm). This classification is based on the work of Gutierrez, Baculi, Pastor, Puma-at, and Balangcod (2013). It was identified that a 50% concentration of Sambong has the greatest antibacterial properties.

pH Level Test

The researcher used the pH test strips to measure the ointment's pH levels, offering insights into its skin compatibility and potential for optimal wound healing. The three (3) samples of 50% concentration of open wound-healing ointment being formulated were tested.

According to Dr. Rodrigues (2023), pH is a measurable parameter between the values of 0 and 14, defining the acidity or alkalinity of a solution. Solutions with a pH = 7 are considered neutral, pH < 7 are considered acidic, and pH > 7 are considered basic or alkaline. The term pH can be defined as the "potential of the hydrogen ions," and its value determines many factors in the chemical environment of molecules, affecting properties such as the solubility of compounds, chemical reactions, availability of nutrients, biological functions, and microbial activity, among many others.

D. In Vivo Test

D.1 Testing the Irritation of the Formulated Open Wound-Healing Ointment (Dermatological Effect Test)

In determining the irritation caused by the formulated open wound-healing ointment using Sambong leaf extracts, the researchers utilized the patch test method. In successfully doing this method, the researchers first gathered all the needed materials such as alcohol, patches, tissue papers, plasters, and scissors. Second, they determined ten (10) respondents from the STEM 12 strand specifically Grade 12 Callidus for the experimental/treatment group, encompassing 5 boys, and Grade 12 Wizard for the control group, composed of 5 boys, whose parents voluntarily signed the consent to allow their children to participate in the study's testing.

Furthermore, the patch test commenced by cleaning/disinfecting the side of the neck area of the respondents using alcohol with cotton balls. Then, the researchers applied a thick layer of Betadine ointment for the positive control while for the experimental/treatment group, it was the formulated open wound-healing ointment on the surface of the respondents' neck. Next, they applied the patches. Once these steps were executed properly, they instructed the respondents not to remove the patches and secure them for 48 hrs. After the period of waiting, the researchers observed the respondents' skin for possible signs of irritation or itchiness.

According to Ludmann (2021), a patch test is a skin test used to find the cause of a possible allergic reaction on the skin. This reaction is called allergic contact dermatitis. Contact dermatitis is a reaction to something that comes into contact with the skin. This kind of allergic reaction usually causes inflammation (redness, itching). Further, the patches placed on the skin during the test were left for 48-hour observation.

Preparation of the Test Organism

The study was conducted following the approved protocol of the Institutional Animal Care and Use Committee (IACUC) and the rules stipulated in the Department of Agriculture Administrative Order No. 40 for the promulgated rules and regulations on the conduct of scientific procedures using animals (Department of Agriculture, 1999).

D.2 Testing the Wound-Healing Properties of the Formulated Ointment in Mice

In vivo testing, the researcher tested the open wound-healing properties of the formulated ointment using the Sprague Dawley Rats (*Rattus norvegicus*) (living organisms) as the vertebrate animal research subjects, that were incised, observed, and treated with both the formulated open wound-healing ointment with 50% concentration (treatment) and Povidone-iodine or Betadine ointment as the positive control and distilled water as negative control. This test lasted for 12 days before the analysis of the results was interpreted.

D. 3 Testing the Tensile Strength

To evaluate the healing efficacy and mechanical integrity of the repaired tissue of the sample mice from treatment and controls, a tensile strength procedure was done. Tensile strength, defined as the force required to separate healing skin, was employed to evaluate the extent of wound healing. The experimental setup comprised fixed shelves with a table. The researchers securely positioned the sample mice on the tensile testing platform, utilizing two Allis forceps attached to either end of the wound, with a rope connected to an empty small cardboard box that contained mini zip lock bags filled with weights. On the tenth day post-wounding, each mouse was anesthetized using a combination of Lidocaine HCl + Epinephrine to ensure the animal's stability on the table. The researchers followed the guidelines from the Albert Einstein College of Medicine regarding Anesthesia and Analgesia in Mice to ascertain the correct dosage based on each mouse's weight. The two forceps were applied firmly 1 cm away from the healed tissue on the incised area of the skin, facing each other.

Subsequently, tap water was added to the mini zip lock bags to gradually increase the weight placed on the small cardboard box, exerting pressure on the wound site and pulling the wound edges apart. The addition of water to the zip lock bags was halted when the wound gaping became apparent, and the total volume of water collected in the mini zip lock bag was measured and recorded. The percentage of tensile strength for the extract and reference drug was then calculated concerning the negative and positive controls treated with a simple ointment (SO) using the specified formula.

On the 10th day post-wounding, each mouse was anesthetized using Lidocaine HCl + Epinephrine, following the Albert Einstein College of Medicine's guidelines for dosage based on weight. The forceps were applied 1 cm from the healed tissue on the incision, facing each other. Water was then added to the zip lock bags, gradually increasing the weight to pull apart the wound edges. When wound gaping occurred, the addition of water was stopped, and the volume collected in the bags was noted.

The percentage of tensile strength for the extract and reference drug, compared to the negative and positive controls treated with a simple ointment (SO), was measured using the formula:

According to Sullivan and Statler (2008), the tensile strength of a wound is a factor in the healing process. Tensile strength is defined as the greatest force a substance can bear without tearing apart. The tensile strength of a wound is directly related to time and is very low for approximately the first 3 weeks following an injury and a primary closure. Extreme care must be taken to protect

the newly formed scar from reinjury at this time. The strength of the wound increases rapidly during the early stages of the maturation phase as the collagen fibers are rearranged and simplified according to the mechanical stresses applied to the scar.

E. Euthanasia of the Mice

In this study, the vertebrate animals were cared for during the procedure. Euthanasia took place away from public view. Further, the researchers used Carbon dioxide (CO₂) inhalation as an effective method for euthanizing rodents, following the procedures stipulated in the Policy for Euthanasia and Carcass Disposal by the Institutional Animal Care and Use Committee (IACUC) (2021). Adherence to ethical guidelines and involving qualified personnel, such as veterinarians or experienced researchers, are essential for proper implementation. Prioritizing animal welfare allows for valuable research while maintaining compassion.

F. Carcass and Biohazards Disposal

All the carcasses were placed in body bags and properly labeled with the method used to ensure death, date, and initials of persons disposing of the carcass. Moreover, in experimenting, all waste generated during the experiment, including used cultures, contaminated materials, and excess ointment, was disposed of according to institutional guidelines for biohazardous waste. Liquid waste was sterilized before disposal, and solid waste was incinerated or autoclaved to prevent environmental contamination.

Variables of the Study

The independent variable of the study is the formulated open-wound-healing ointment using Sambong (*Blumea balsamifera*) leaf extracts with Guava (*Psidium guajava*) scent, while the dependent variables are the antibacterial properties, pH level, irritation test, wound-healing properties, and tensile strength. On the other hand, the control variable of the study is Mupirocin and Betadine ointment.

Data Gathering Techniques

The researcher observed which wounds in both control and treatment groups at a 50% concentration healed faster and were the most effective. Consequently, she determined which of the control and treatment groups healed quickly by measuring the wound area on the 2nd, 4th, 6th, 8th, 10th, and 12th days. The researcher also followed a formula to determine the optimal treatment for healing wounds (Demilew, Adinew, & Asrade, 2018).

$$\% \text{ of Wound Closure} = \frac{\text{Original Wound Area (Day 1)} - \text{Specific Day Wound Area}}{\text{Original Wound Area (Day1)}} \times 100$$

Original Wound Area (Day1)

Statistical Analysis

The acceptance or rejection of the null hypothesis presented in this study was determined using Analysis of Variance (ANOVA) -Two Factors with Replication in identifying the significant difference in the antibacterial properties, the mean body weight of sample mice, the size of wounds, and tensile strength. All tests were done at a 0.05 level of significance.

IV. RESULTS AND DISCUSSION

Formulating an Open Wound-Healing Ointment

Sambong leaf extraction involved gathering leaves from diverse locations, cleaning, dehydrating, and extracting with ethyl alcohol. The resulting pure crude extract was refined and combined with an ointment base for evaluation, yielding a 50% concentration. The specific measurements of formulation included Beeswax (24g), Olive Oil (48.12g), Coconut Oil (48.18g), and Honey (10.98g).

IN VITRO TESTING

Antibacterial Testing of Sambong Leaf Extracts

The researchers evaluated the antibacterial properties of Sambong (*Blumea balsamifera*) leaf extracts using the Disk Diffusion Method, where agar plates were inoculated with a standardized inoculum of the test microorganism, i.e., *Staphylococcus aureus*. Then, filter paper disks (about 6 mm in diameter) containing the test compound at the desired concentration. The treatment included 50% Sambong (*Blumea balsamifera*) concentration, while controls comprised Distilled Water (NC) and Mupirocin (PC). Zones of inhibition (ZOI) were measured in millimeters, categorized as inactive (0-9 mm), partially active (10-13 mm), active (14-19 mm), or very active (>19 mm). Table 1 shows the outcomes of the comparative measures of the zones of inhibition of the treatment and controls.

Table 1. Comparative Measures of the Zones of Inhibition of the Treatment and Controls against *S. aureus*

SAMPLES OF CONCENTRATION	DISK 1	DISK 2	DISK 3	AVERAGE	STATUS OF ANTIBACTERIAL PROPERTIES
50% Sambong Leaf Extracts	9	10	10	9.67	Partially Active
Positive Control (Mupirocin)	7	7	7	7.00	Inactive
Negative Control (Distilled Water)	0	0	0	0.00	Inactive

Table 1 presents a comparison of the antibacterial activity of 50% Sambong leaf extract, a positive control (Mupirocin), and a negative control (Distilled Water) against *Staphylococcus aureus*. The results are measured by the zones of inhibition (in millimeters) around each sample disk, with three trials for each.

The results in the measurement of the zone of inhibition showed that 50% (*Blumea balsamifera*) leaf extracts inhibited *S. aureus*. The average results of the three (3) replicates indicated **Partially Active** as evidenced by their ZOI measurements of 9, 10, and 10 in mm, respectively. This suggests that Sambong leaf extract does exhibit some level of inhibition against *S. aureus*, though it is not as strong as more potent antibacterial agents.

In Positive Control (Mupirocin), all three trials showed a zone of inhibition of 7 mm, with an average of 7.00 mm. Surprisingly, it is marked as **Inactive** despite having measurable zones of inhibition. This might indicate that the threshold for activity was set higher than 7 mm, or it could suggest that Mupirocin's efficacy against *S. aureus* in this specific experimental setup was suboptimal.

In Negative Control (Distilled Water), no zones of inhibition were observed, with a 0.00 mm average. As expected, it is labeled as **Inactive**, confirming that distilled water does not possess antibacterial properties.

The results of antibacterial property tests imply that Sambong leaf extract has potential as an antibacterial agent. The partial activity demonstrated by the Sambong leaf extract suggests it has some antibacterial potential against *S. aureus*. While not highly potent, it could be explored further, perhaps with higher concentrations or in combination with other antibacterial agents, to assess if its efficacy can be enhanced.

Moreover, the inactivity label for Mupirocin, despite some inhibition, could imply limitations in the experimental conditions or resistance of the *S. aureus* strain used. Further testing might be necessary to confirm these findings and determine if the strain is resistant to Mupirocin or if a higher concentration would yield better results. On the other hand, the lack of inhibition with distilled water confirms the validity of the experimental design, as any observed zones of inhibition are likely due to the active components in the test samples rather than contamination or external factors.

Simply put, while Sambong leaf extract shows promise as a natural antibacterial agent, additional research would be needed to understand its potential fully. The unexpected result with Mupirocin may also warrant further investigation, especially given its common use as an antibiotic against *S. aureus*.

These results are confirmed by Sakee, Maneerat, Cushnie, and De-eknamkul (2011), who conducted a study revealing that extracts derived from *B. balsamifera* exhibited activity against a variety of infectious microorganisms as well as those capable of producing toxins. The researcher suggests that the active components found in this plant offer potential for future development in combating microbial diseases, either through treatment or prevention measures.

pH Level Testing of the Formulated Open Wound-Healing Ointment

This study tested the pH level of the formulated open wound-healing ointment concentration. Using pH test strips, the ointment's pH levels were measured, offering insights into its skin compatibility and potential for optimal wound healing. The results in Table 2 below indicate the pH level of the ointment concentration.

Table 2. pH Level Results of the Formulated Ointment

pH LEVEL TESTING		
TRIAL	SAMPLE	RESULTS
1	50% Concentration	6
2	50% Concentration	5
3	50% Concentration	5
AVERAGE		5.33

Table 2 shows the pH levels recorded for a 50% concentration of the ointment across three trials. Analysis of results elucidates that when it comes to acidity level, the pH values fall between 5 and 6, indicating that the ointment is mildly acidic. Healthy human skin typically has a pH range of about 4.5 to 5.5, so a pH of 5.33 is close to the skin's natural acidity, which is beneficial for maintaining the skin barrier and preventing bacterial growth. Moreover, the pH levels across the three trials are consistent, with only a slight variation, suggesting that the formulation is stable in terms of its pH.

Considering the pH test results, it implies that the 50% concentration of Sambong leaf extract has skin compatibility. The mildly acidic pH (5.33) is within a range that is generally compatible with the skin's natural pH. This suggests that the ointment would be less likely to cause irritation or disrupt the skin barrier, making it suitable for wound application and use on sensitive or damaged skin. Also, there is potential for antimicrobial activity since the slightly acidic pH may contribute to creating an environment that discourages the growth of harmful bacteria. Many pathogenic bacteria thrive in neutral to slightly alkaline conditions, so a pH of 5.33 could help prevent infections on open wounds.

Furthermore, it is likely good for the preservation of the skin barrier. An ointment with a pH similar to the skin's natural acidity helps preserve the skin barrier. This is important for wound healing as a stable skin barrier supports faster and safer recovery. Additionally, it promotes market appeal. Products with a skin-friendly pH may appeal to consumers seeking natural and gentle options for wound care. The pH level aligns with dermatological guidelines for products that are safe for sensitive skin.

In summary, the mildly acidic pH of the ointment makes it a promising option for wound healing, as it aligns well with the skin's natural pH, minimizes irritation risk, and may offer some protection against microbial infections. These factors enhance its suitability for broader dermatological applications and increase its potential appeal in the market as a safe, skin-friendly ointment.

Certainly, the pH scale establishes an object's acidity or basicity. The scale runs from 0 to 14, with neutrality represented by 7. pH values under 7 indicate acidity, whereas pH values above 7 suggest alkaline (Water Science School, 2019). Anything higher is alkaline, anything lower is acidic, and the skin's pH is weakly acidic, so the best products to use on your skin should have a comparable pH. However, the skin frequently has a wide pH range, with an acidity of between 4 and 7 (Cherney, 2022). When your skin becomes overly alkaline, it becomes dry and sensitive. You may also notice inflammation, wrinkles, and sagging (NewGelPlus, 2020). However, if it is overly acidic, you are more likely to develop inflammatory skin diseases such as acne and eczema (Mukherjee, 2019).

IN VIVO TESTING

In Vivo testing is a crucial step in the formulation of wound-healing ointment, as it provides valuable information on the ointment's safety, efficacy, pharmacokinetics, and suitability for a range of wound types and severities.

Dermatological Test of the Formulated Ointment

This study conducted a dermatological effect test through a patch test of the formulated ointment using Sambong (*Blumea balsamifera*) leaf extracts. This test was a diagnostic exam that the researcher used to determine whether the formulated ointment resulted in skin irritation or an allergic reaction. It utilized 5 students from STEM 12 Callidus (Experimental/Treatment Group) and 5 students from STEM 12 Wizard (Control Group) who willingly participated to be the subjects of the testing. The formulated ointment was applied to their necks for 48 hours of observation. Table 3 on the next page shows the results of the patch test.

Table 3. Results of Dermatological Effect Test (Patch Test)

RESPONDENTS	CONTROL	RESPONDENTS	TREATMENT
R1	1	R1	1
R2	1	R2	1
R3	1	R3	1
R4	1	R4	1
R5	1	R5	1

Legend: 1 denoting the absence of irritation, 2 denoting the presence of irritation

The table shows data on whether respondents experienced irritation from a control sample and a treatment sample. It lists five respondents (R1 to R5) and their reactions to both a control and a treatment sample. The legend indicates that a value of "1" denotes the absence of irritation, while "2" denotes the presence of irritation. All respondents (R1 to R5) show a value of "1" for both the control and treatment samples, indicating that none of them experienced irritation from either sample.

Since all values are "1," it suggests that the treatment (likely the formulated organic wound ointment being tested) did not cause skin irritation for any of the respondents in this sample group. The absence of irritation in both the control and treatment groups implies that the treatment is as safe as the control, which may not contain active ingredients, and suggests good dermatological compatibility.

These results indicate that the formulated organic wound healing ointment using Sambong with Guava scent is non-irritating for the skin of all respondents in the test. This is a positive outcome for its dermatological safety and suggests it could be suitable for individuals with sensitive skin. This finding supports the potential for safe, irritation-free application of the ointment on open wounds, which is crucial for its acceptance and effectiveness in the community. Also, this may increase consumer acceptance of natural or organic formulations and encourage the use of products containing botanical extracts (Ong, Maibach, & Simion, 2014).

Furthermore, the absence of irritation in all respondents highlights the ointment's potential for broader dermatological applications beyond wound healing. This suggests that it may also be suitable for individuals with various skin types, including those prone to allergies or sensitivity. The use of natural ingredients like Sambong and Guava scent could further enhance consumer confidence in the product's safety and efficacy. Additionally, this aligns with the growing demand for organic and eco-friendly skincare solutions, which prioritize both health benefits and environmental sustainability. Future studies may explore its long-term effects and potential enhancements to maximize its therapeutic properties.

Area of Wound

The wound area was measured using a caliper, a precision tool in biomedical research. Observations and data collection were consistent during the application of 50% concentration, Positive Control (Betadine Ointment), and Negative Control (Distilled Water). Table 4 below shows the comparative results of the wound area.

Table 4. Area of Wound

		AREA OF WOUND (in mm ²)						
VARIABLES		DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12
50% Concentration	R1	94.25	133.52	113.09	81.68	42.41	15.71	3.14
	R2	241.78	276.46	131.95	120.95	47.12	27.49	18.85
	R3	65.97	38.48	38.48	27.49	12.57	0.79	0.20
	AVERAGE	134.00	149.49	94.51	76.71	34.03	14.66	7.40
POSITIVE CONTROL (Betadine Ointment)	R1	102.10	120.95	98.96	62.83	54.98	18.85	3.93
	R2	226.98	82.47	76.97	60.48	28.27	6.28	2.36
	R3	56.55	28.27	9.42	11.78	4.71	3.14	2.36
	AVERAGE	128.54	77.23	61.78	45.03	29.32	9.42	2.88
NEGATIVE CONTROL (Distilled Water)	R1	81.68	77.75	70.69	42.41	12.57	15.71	12.57
	R2	122.52	267.04	164.93	132.73	63.62	37.70	15.71
	R3	103.67	86.39	70.69	23.56	12.57	9.42	14.14
	AVERAGE	102.62	143.73	102.10	66.23	29.59	20.94	14.14

Table 4 presents data on the area of wounds (in mm²) measured over 12 days for three groups: a 50% concentration treatment, a positive control (Betadine ointment), and a negative control (distilled water). Each row shows measurements for individual respondents (R1, R2, and R3), along with an average for each group on each day. The results indicate a gradual reduction in wound size over time for all groups. The 50% Sambong treatment yielded an average wound area of 7.40 mm² (94.48% of wound-healing), while Betadine ointment showed 2.88 mm² (97.76% of wound-healing) and Distilled Water displayed 14.14 mm² (86.22% of wound-healing).

For the 50% Concentration (Treatment), the average wound area decreases consistently over the 12 days, starting from 114.80 mm² on Day 0 and reducing to 7.40 mm² by Day 12. This group shows a significant reduction in wound area, suggesting that the 50% concentration treatment is effective in accelerating wound healing. On the other hand, in the Positive Control (Betadine Ointment), similar to the 50% concentration, the average wound area decreases over the 12 days, from 123.54 mm² on Day 0 to 2.88 mm² by Day 12. Betadine appears to be very effective, achieving the smallest average wound area by the end of the study. Consequently, the Negative Control (Distilled Water) group shows the slowest reduction in wound area, starting from 102.62 mm² on Day 0 and only decreasing to 14.14 mm² by Day 12. This group's slower wound healing compared to the other two groups suggests that distilled water does not aid the wound healing process as effectively as the 50% concentration treatment or Betadine.

For comparative analysis, when it comes to effectiveness, both the 50% concentration treatment (formulated organic open wound-healing ointment) and the positive control (Betadine) significantly reduced wound size, with Betadine showing a slightly faster healing effect by Day 12. However, the 50% concentration still demonstrated a substantial reduction in the wound area. For the negative control performance, the distilled water group, as expected, showed slower wound healing, which validates the effectiveness of both the 50% concentration and Betadine ointment.

Simply, these results suggest that the formulated organic ointment at 50% concentration is effective in promoting wound healing, though slightly less effective than Betadine. Nonetheless, it significantly outperformed the negative control, indicating its potential as a viable wound treatment option, particularly for individuals seeking an organic alternative. Indeed, Sambong (*Blumea balsamifera*) is a well-known herbal flowering plant native to the Philippines, cherished for its medicinal attributes in addressing a range of health issues, including wound healing, rheumatism, diarrhea, and spasms, as well as symptoms associated with colds and coughs. Moreover, it is traditionally employed for treating infected wounds and respiratory infections and easing stomach discomforts (Boy, Rutilla, Santos, Ty, Yu, Mahboob, Tangpoong, & Nissapatorn, 2019).

Body Weight Measurement

The research included monitoring mice's body weight before incision and every other day during treatment application. An analytical balance was used to track weight changes. Data on mice's body weight measurements are provided in the accompanying table.

Table 5. The Weight of *Rattus Norvegicus* (in g) During Observation (Every Other Day Measurement)

		BODY WEIGHT OF MICE (in grams)						
VARIABLES		DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12
50% Concentration	R1	23.37	24.48	25.75	24.18	24.18	25.04	23.11
	R2	21.43	14.67	20.68	20.4	21.82	21.86	17.71
	R3	22.9	23.96	23.35	20.62	19.99	22.33	21.45
	AVERAGE	22.57	21.04	23.26	21.73	22.00	23.08	20.76
POSITIVE CONTROL (Betadine Ointment)	R1	24.45	22.63	26.94	26.40	23.77	22.82	24.47
	R2	24.43	21.86	25.86	25.54	21.21	25.38	23.25
	R3	23.55	23.18	26.76	22.79	24.97	25.90	26.17
	AVERAGE	24.14	22.56	26.52	24.91	23.32	24.70	24.63
NEGATIVE CONTROL (Distilled Water)	R1	21.14	23.05	30.79	29.70	30.26	31.36	33.17
	R2	26.46	22.51	29.00	29.40	28.56	29.03	25.62
	R3	31.56	32.78	29.15	32.52	28.70	28.48	29.41
	AVERAGE	26.39	26.11	29.65	30.54	29.17	29.62	29.40

Some mice exhibited reduced food intake during the epithelialization phase but returned to normal eating habits later. In vivo testing on *Rattus norvegicus* showed varied weight results. After the 12 days, the 50% Sambong treatment group had an average weight of 20.76g, a 1.81g difference from the initial measurement. The Positive Control (Betadine) group averaged 24.63g, showing a 0.49g difference, while the Negative Control (Distilled Water) group averaged 29.4g, with a 3.01g difference.

Concerning the results of the body weight measurement of the mice samples, 50% concentration treatment shows their weights fluctuate slightly over time, but there is a slight decrease in average weight from 22.57g on Day 0 to 20.78g on Day 12, suggesting some possible weight loss due to this treatment. On the other hand, in the Positive Control (Betadine Ointment) group, their weights also fluctuated, with a slight initial weight increase by Day 4. However, by Day 12, the average weight was 24.63g, close to the starting average of 24.14g. This suggests that the Betadine ointment might have a neutral or stabilizing effect on weight over time. Additionally, for the Negative Control (Distilled Water), this group shows an overall trend of weight increase, with the average starting at 26.39g and reaching 29.40g by Day 12. The distilled water appears to have allowed natural growth or maintenance without any adverse effects.

By these data, the researcher observed that the weight loss in 50% concentration, the mice treated showed a slight downward trend in weight, which could indicate a side effect of the treatment. Conversely, there is a stable weight in Positive Control (Betadine), which means that it does not seem to induce significant weight changes, implying it has little to no adverse impact on the mice's overall health in terms of body weight. Furthermore, the weight gain in Negative Control suggests that the treatment-free group maintained normal growth.

This is confirmed during the measurement of body weight in each mouse where the researcher noted that some mice exhibited reduced food intake, resulting in daily fluctuations in their weights, which led to the results that some mice showed an increase in weight, while others demonstrated a decrease. Thus, it implies that the 50% concentration treatment might negatively impact body weight, whereas Betadine has a neutral effect. The distilled water group acts as a baseline, showing natural weight gain without treatment influence.

However, according to the study conducted by Vijayaraghavan, Rajkumar, and Seyed (2017), after injury, mice often experience an acute inflammatory response characterized by the recruitment of immune cells to the site of injury. This immune response can cause a localized increase in tissue swelling and fluid accumulation, which can result in weight gain. As the wound begins to heal, the inflammation typically subsides, and fluid accumulation decreases, leading to a decrease in weight.

Tensile Strength Testing

The tensile strength test evaluated tissue resilience to mechanical stress, providing insights into the quality of healed tissue post-intervention. Table 6 below shows the results of the tensile strength.

Table 6. Tensile Strength of Wound Healed (in g/mm²)

TENSILE STRENGTH OF WOUND HEALED (in g/mm ²)				
SAMPLES OF CONCENTRATION	Replicate 1	Replicate 2	Replicate 3	AVERAGE
50% Sambong Leaf Extracts	26.03	16.96	177.18	73.39
Positive Control (Betadine Ointment)	7.54	11.85	21.14	13.51
Negative Control (Distilled Water)	20.83	4.13	6.02	10.33

The table displays the tensile strength of healed wounds (in g/mm²) for three groups: a 50% concentration of Sambong leaf extract, a positive control (Betadine ointment), and a negative control (Distilled Water). Each group has three replicates, along with an average tensile strength calculated across these replicates. Analysis of Results revealed that in 50% Sambong Leaf Extract, the tensile strength of healed wounds in this group shows a high average of 73.39 g/mm². This group has the highest variability, with measurements ranging from 16.96 g/mm² to 177.18 g/mm² across the replicates. Despite the variability, the high average suggests that the Sambong leaf extract promotes strong wound healing.

Moreover, in Positive Control (Betadine Ointment), the average tensile strength is 13.51 g/mm^2 , which is significantly lower than the Sambong leaf extract group. Betadine shows more consistent results across replicates, with values ranging from 7.54 g/mm^2 to 21.14 g/mm^2 . Although Betadine is effective for wound healing, as indicated in the previous table, it does not result in as strong a healed wound as the Sambong leaf extract.

On the other hand, in Negative Control (Distilled Water), the average tensile strength for the distilled water group is 10.33 g/mm^2 , which is the lowest among the three groups. This group also shows a wide range of values, from 4.13 g/mm^2 to 20.83 g/mm^2 . The low tensile strength confirms that distilled water does not significantly promote wound healing strength, serving as a baseline for comparison.

The results of the tensile strength test show the effectiveness of Sambong leaf extract. The high average tensile strength in the Sambong leaf extract group indicates that it not only promotes healing but also results in stronger healed tissue compared to the Betadine ointment and distilled water. This suggests that Sambong leaf extract may enhance collagen formation or other factors contributing to tissue strength. While Betadine is effective in reducing wound size, it does not appear to provide the same level of tensile strength as the Sambong leaf extract. This could imply that while Betadine aids in closing wounds, it may not support the same degree of structural integrity in the newly formed tissue.

Furthermore, the results imply the potential for organic treatment. Sambong leaf extracts at a 50% concentration could serve as a potent alternative to conventional wound care treatments like Betadine, offering both effective healing and enhanced wound strength. This is particularly important for patients who may need durable wound healing, such as those at risk of re-injury. In summary, these findings underscore the potential benefits of using Sambong leaf extract in wound care, both for its healing capabilities and for producing stronger healed tissue.

According to Raval (2019), tensile strength in wound healing is crucial because it represents the ability of the wound tissue to resist tearing or breaking under stress. As a wound heals, its tensile strength progressively increases, indicating that the tissue is getting stronger and more stable, which is essential for the wound to withstand normal daily activities and prevent re-injury.

Difference Between the Treatment and Controls Regarding their Antibacterial Properties, the Mean Body Weight of Sample Mice, Size of Wounds, and Tensile Strength

This study subjected the treatment, i.e., Sambong (*Blumea balsamifera*) leaf extracts and controls (Betadine Ointment for positive and Distilled Water for negative) to statistical analysis to determine their significant difference in terms of the zone of inhibitions they demonstrated during the in vitro testing using the Disk Diffusion Method. Using the Analysis of Variance (ANOVA) -Two Factors with Replication, Table 7 shows the results yielded.

Table 7. Difference Between Treatment and Control in Terms of Antibacterial Properties

Source of Variations	SS	df	MS	F	P-value	F crit
Rows	149.5556	2	74.77778	673	0.00	6.944272
Columns	0.222222	2	0.111111	1	0.444444	6.944272
Error	0.444444	4	0.111111			
Total	150.2222	8				

The table above shows the results of the difference between treatment and control in terms of antibacterial properties among the variables with a p-value of 0.00 ($F=6.94$). Thus, there is a significant difference among the variables in terms of antibacterial properties presented with a p-value of $0.00 < 0.05$, where 0.05 is the level of significance.

The significant difference in antibacterial properties among the treatments (as shown by the low P-value for the "Rows" factor) implies that at least one of the treatments has a markedly different level of antibacterial activity. Given the results in the previous table, it is likely that the 50% Sambong leaf extract demonstrates a stronger antibacterial effect compared to Mupirocin and Distilled Water. The significant result supports the possibility that Sambong leaf extract could be a viable antibacterial agent, warranting further investigation into its use against *S. aureus* and potentially other bacterial strains.

Furthermore, the non-significant result for "Columns" suggests consistency across trials or other conditions in the experiment, reinforcing the reliability of the observed differences due to treatment effects alone. Hence, the ANOVA results confirm that there is a meaningful difference in antibacterial effectiveness among the treatments, particularly highlighting the partial activity of Sambong leaf extract as observed in previous measurements. This finding could prompt further research into optimizing the concentration or combining Sambong extract with other agents to enhance its antibacterial potential.

Additionally, the consistency across trials suggests that external factors had minimal influence on the results, strengthening the validity of the experiment. This reliability is essential for future studies aiming to refine the formulation and assess its broader applicability. Exploring synergistic effects with other natural antibacterial agents could further enhance the efficacy of Sambong extract. Moreover, identifying the specific bioactive compounds responsible for its antibacterial activity could lead to more targeted applications in medicine and skincare. Ultimately, these findings support the potential of plant-based alternatives in developing effective and sustainable antibacterial treatments.

Table 8. Difference Between Treatment and Control In terms of Mean Body Weight of Sample Mice

Source of Variation	SS	df	MS	F	P-value	F crit	Remarks
Sample (Variable)	475.9937	2	237.9968	32.75831	0.00	3.219942	Significant
Columns (Day)	61.90471	6	10.31745	1.420112	0.23	2.323994	Not Significant
Interaction	37.13219	12	3.094349	0.425912	0.94	1.991013	Not Significant
Within	305.1399	42	7.265237				
Total	880.1705	62					

Table 8 shows the results of the difference between treatment and control in terms of the Mean Body Weight of Sample Mice with a p-value of 0.00 ($F=3.22$). There is a significant difference among the variables presented with a p-value of $0.00 < 0.05$ in terms of the mean body weight of sample mice, where 0.05 is the level of significance.

The significant difference across treatments (Sample variable) suggests that the type of treatment administered to the mice significantly influences their body weight. This could mean that certain treatments either promote weight gain or loss in mice, indicating physiological effects beyond antibacterial action. The non-significant result for "Columns (Day)" suggests that the passage of time, or the duration over which the experiment was conducted, does not have a significant impact on body weight. This could mean that the treatment effects on body weight are immediate or do not vary greatly over time.

Moreover, the absence of a significant interaction effect means that the influence of the treatments on body weight remains consistent across different days. This consistency implies that the treatments have stable effects on body weight, regardless of the duration they are administered over the observed period.

Thus, the findings suggest that the different treatments lead to significant variations in body weight among the mice. However, these effects are stable over time, as neither the duration nor the interaction between treatment and time significantly impacts the results. Hence, the treatment and control tested may have physiological effects, potentially valuable or concerning, depending on the experimental goals. This result highlights the need for further investigation into the treatment's (50% Sambong leaf extract) broader physiological impacts, as changes in body weight could indicate metabolic effects that may affect the overall health of the subjects.

Table 9. Difference Between the Treatment and Control in Terms of the Size of the Wound

Source of Variation	SS	df	MS	F	P-value	F crit	Remarks
Sample (Variables)	5880.916	2	2940.458	1.004043	0.00	3.219942	Significant
Columns (Day)	125749.1	6	20958.19	7.156343	0.00	2.323994	Significant
Interaction	10232.34	12	852.6953	0.29116	0.00	1.991013	Significant
Within	123001.9	42	2928.617				
Total	264864.3	62					

The table above shows the results of the difference between treatment and control in terms of the size of the wound with a p-value of 0.00 ($F=3.22$). Therefore, there is a significant difference in the size of wounds from day 0 to day 12 within the variable as p-value $0.00 < 0.05$, where 0.05 is the level of significance.

The "Sample (Variables)" row has an F-value of 1.00 and a p-value of 0.00, marked as "Significant." This suggests that there is a statistically significant difference in wound size between the treatment and control groups. It implies that the treatment has an impact on wound size, possibly enhancing wound healing in comparison to the control. Clinically, this could suggest that the treatment is effective in reducing wound size, making it a potentially valuable intervention.

Furthermore, the "Columns (Day)" variable has a p-value of 0.00 and an F-value of 7.15, also marked as "Significant." This indicates that wound size significantly changes over the days studied (from day 0 to day 12). This result demonstrates that wound size naturally decreases over time, reflecting the normal healing process. The significant day effect highlights the importance of time in wound healing, as wounds typically reduce in size as they heal. This result aligns with expected biological healing and supports the idea that, regardless of treatment, time is a crucial factor in wound recovery.

Additionally, the interaction between "Sample (Variables)" and "Columns (Day)" has an F-value of 0.29 with a p-value of 0.00, marked as "Significant." This means that there is a statistically significant interaction between the treatment type and time, indicating that the effect of the treatment on wound size varies across different time points. Hence, the significant interaction suggests that the treatment has a different impact on wound size reduction at various time points. For example, the treatment may accelerate healing initially, with effects that taper off over time, or it may show more pronounced effects at specific stages. This finding could be clinically relevant as it indicates that the timing of the treatment plays a role in its effectiveness, and future studies might explore optimal timing for the best outcomes.

In sum, the significant difference between treatment and control, along with the interaction effect, suggests that the treatment positively impacts wound healing. These findings support the treatment as a potentially effective intervention for reducing wound size. Clinicians might consider using this treatment, especially if timing can be optimized to maximize its effects. Similarly, the significant day effect highlights that time alone is a critical factor in wound healing. While the treatment appears effective, this result reinforces the importance of allowing sufficient healing time, with or without intervention.

Moreover, the significant interaction between treatment and time points to the potential benefits of optimizing treatment timing. Future research may examine whether administering the treatment at specific times yields better results or explore the exact pattern of wound size reduction over time.

Table 10. Difference Between the Treatment and Control in Terms of Tensile Strength

Source of Variation	SS	df	MS	F	P-value	F crit	Remarks
Rows	7572.732	2	3786.366	1.422087	0.341569	6.944272	Not Significant
Columns	5813.388	2	2906.694	1.091699	0.418471	6.944272	Not Significant
Error	10650.17	4	2662.542				
Total	24036.29	8					

There is no significant difference among the tensile strength before, during, and after the treatment among the variables as p-value $0.41 > 0.05$, where 0.05 is the level of significance.

The F-statistic for the rows is 1.42 with a P-value of 0.34 , which is greater than the significance level typically set at 0.05 . The F critical value (6.94) is higher than the calculated F value, indicating that the treatment effect is not statistically significant. This suggests that there is no significant difference in tensile strength due to the variation in treatments, implying that changing treatments might not impact tensile strength.

Moreover, the F-statistic for the columns is 1.09 , with a P-value of 0.42 , which also exceeds the 0.05 threshold. The calculated F is again lower than the critical F value of 6.94 , indicating that the control variation does not have a statistically significant effect on tensile strength. This result implies that any variation in controls does not significantly impact tensile strength, suggesting that different control levels are not influencing the outcome.

Since neither the treatments nor controls show significant effects on tensile strength, the results indicate that tensile strength remains consistent across the tested variations. This could imply that factors beyond those analyzed may influence tensile strength or that the experimental conditions did not have enough impact to show statistical significance.

Simply put, the results suggest that neither the treatments nor controls significantly impact tensile strength under the conditions tested. Further investigations or different variable selections might be necessary to identify influential factors.

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