



Potential Wound Healing Effect of Ethanolic Leaf Extract of *Sandoricum koetjape* (Meliaceae) in Excision Wound Model in Swiss Albino Mice

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ABSTRACT

This study assessed the potential wound-healing effect of ethanolic leaf extract of *Sandoricum koetjape* (Meliaceae) using an excision wound model in Swiss albino mice. Twenty- four mice were distributed equally into four treatment groups: negative control ointment, positive control ointment, 5% *S. koetjape* extract ointment, and 10% *S. koetjape* extract ointment. Wound area was measured on Day 0, Day 3, Day 7, and Day 14, while wound contraction was evaluated on Day 3, Day 7, and Day 14 using Day 0 as the baseline reference. Descriptive statistics, a linear mixed-effects model, day-specific group comparisons, and post hoc tests were used to evaluate visible wound closure and treatment differences. Mean wound area decreased across the observation period in all groups. By Day 14, the remaining mean wound areas were 0.61 mm² for the negative control group, 0.55 mm² for the positive control group, 0.07 mm² for the 5% extract group, and 0.00 mm² for the 10% extract group. Mean wound contraction also increased over time, reaching 97.85%, 98.05%, 99.75%, and 100.00%, respectively, by Day 14.

The overall mixed-effects analysis showed a significant effect of observation day, indicating substantial time-related wound contraction, but the treatment-group effect and treatment group by day interaction were not statistically significant. Dayspecific comparisons showed no significant group differences on Day 3 or Day 7, but a significant group difference on Day 14. Post hoc analysis indicated that only the 10% extract group had significantly greater final wound contraction than the negative control group after adjustment. The extract-treated groups were not statistically superior to the positive control, and the 10% extract group was not significantly different from the 5% extract group. These findings suggest that *S. koetjape* extract ointments, particularly the 10% formulation, demonstrated promising final-stage visible wound-contraction activity relative to the negative control. However, the results do not establish superiority over the standard treatment, a statistically confirmed dose-dependent response, or complete biological wound-healing efficacy beyond wound area reduction and contraction.

Keywords: *Sandoricum koetjape*, Wound Healing, Excision Wound Model, Ethanolic Extract, Swiss Albino Mice, OECD 402, Phytochemicals, Topical Ointment, Povidone Iodine.

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CHAPTER ONE

INTRODUCTION

➤ Introduction

Skin wounds caused by cuts or excisions are common injuries that disrupt the protective barrier of the body and increase the risk of local complications. Timely closure of these wounds is essential to restore tissue integrity and function. Wound healing proceeds through overlapping stages (hemostasis, inflammation, proliferation, and remodeling) that collectively return the skin to continuity (Criollo-Mendoza et al. 2023; Cedillo-Cortezano et al. 2024). In practical settings, the progress of this process can be reflected by observable outcomes such as reduction in wound area and time to complete epithelialization.

Because commercial products may be costly or inaccessible in some communities, there has been interest in affordable, plant-based options for managing skin cuts. Over the past decade, studies have reported that phytochemicals commonly found in medicinal plants (such as flavonoids, tannins, saponins, limonoids, and terpenoids) were associated with improved wound closure in experimental cut/excision models, often observed as faster contraction and earlier closure (Liu et al. 2022; Bailly 2022). These associations supported further investigation of locally available species in simple, standardized models.

Within the Meliaceae family, there was emerging evidence of such woundhealing potential. For example, an in vivo study using the methanol extract of *Trichilia dregeana* leaves (Meliaceae) demonstrated significant wound-healing activity in mice, as evidenced by increased wound contraction, shortened epithelialization period, and improved tensile strength. In addition, its extract exerted anti-inflammatory effects by inhibiting edema and granuloma formation. These outcomes provided direct proof that members of the Meliaceae family possess compounds with wound-healing properties (Shewaye et al. 2023). Thus, this finding suggests that related Meliaceae species may also harbor compounds useful for healing skin cuts through similar mechanisms.

Sandoricum koetjape (Meliaceae), commonly known as Santol, is a tropical tree native to Southeast Asia. Beyond its edible fruit, studies have reported that the leaves and bark contain terpenoids, limonoids, and flavonoids, compounds with documented pharmacological actions that may aid wound healing (Hamzah et al. 2024; Bailly 2022). Despite these promising bioactivities, there was still no in vivo study that specifically investigated the wound-healing potential of *S. koetjape* through scientific validation.

Therefore, this study aimed to explore and determine the potential wound healing properties of *S. koetjape* leaf extract as a natural wound-healing agent using an excision wound model in Swiss albino mice, by comparing wound contraction rate between treatment and control groups. Findings provided baseline experimental evidence to inform whether *S. koetjape* merits further pharmacological investigation for accessible, plant-based care of skin cuts. The results of this study may lead to the development of affordable, plant-derived wound-healing treatment, and encourage further scientific exploration of locally available natural resources.

➤ Background of the Study

Wound healing is a vital biological process that restores the skin's continuity after injury. In the case of cuts or excisions, the skin undergoes a sequence of overlapping stages (hemostasis, inflammation, proliferation, and remodeling) that ultimately result in wound closure (Criollo-Mendoza et al. 2023; Fernandez-Guarino et al. 2023). When this process is delayed, recovery becomes prolonged and the risk of complications increases. For a minor or simple cut, this process typically takes around two weeks to complete its most visible stages, with complete remodeling taking considerably longer (MedlinePlus 2024). The proliferative phase, which generally occurs within the first two weeks, is characterized by the formation of new tissue, including wound contraction, a key process where the wound margins pull inward to reduce the size of the defect (Cardoso et al. 2025).

Conventional wound-care products, such as antiseptics, are widely utilized to promote healing and prevent infection. However, these agents may be costly, limited in availability in low-resource settings, and associated with issues such as local irritation. Antibiotics, meanwhile, are often difficult to access as it is not readily obtainable for wound-management purposes (Alvarez-Peral et al. 2023). This has encouraged interest in medicinal plants as affordable alternatives for wound care. Previous studies report that phytochemicals such as flavonoids, tannins, terpenoids, and saponins contribute to wound healing through multiple mechanisms. Flavonoids provide antioxidant and antiinflammatory effects that minimize tissue damage and swelling. Tannins promote wound contraction and facilitate tissue regeneration by forming protective protein complexes. Terpenoids enhance collagen synthesis, which strengthens the repair of injured tissue, while saponins stimulate angiogenesis and re-epithelialization, accelerating wound closure (Liu et al. 2022; Zhang et al. 2022).

Povidone-iodine, in particular, is a well-established and trusted standard drug in wound healing studies due to its antiseptic properties, favorable safety profile, and established use in preclinical and clinical contexts. It is frequently employed to assess antimicrobial efficacy and tissue-compatibility profiles using in vitro and animal models in preclinical studies, while in clinical practice it is widely used for wound cleansing, infection prevention, and perioperative skin preparation (Monstrey et al. 2023). Given this, a povidone-iodine solution served as the positive control in this study, providing a benchmark for evaluating the wound contraction rate and healing progression of the plant-based treatment.

The Meliaceae family is recognized for its pharmacological potential, particularly its anti-inflammatory, antioxidant, antimicrobial, and tissue-repairing properties. Notably, *Trichilia dregeana* (Meliaceae) demonstrated significant woundhealing activity in an in vivo study, where its leaf extract enhanced wound contraction, reduced epithelialization time, and improved tensile strength, alongside antiinflammatory effects (Shewaye et al. 2023). These findings suggest that related members of the Meliaceae family may also contain compounds relevant to cut wound repair.

Sandoricum koetjape, commonly known as Santol, is a tropical tree native to Southeast Asia and widely utilized in folk medicine for conditions such as fever, diarrhea, and skin diseases (Wijaya 2022). Traditionally, in countries like the Philippines, Indonesia, Malaysia, and Thailand, its leaves and bark are applied as poultices for cuts and abrasions, used in decoctions for washing small wounds, or made into hot compresses for soft-tissue recovery, reflecting its role in promoting wound healing (Agapin 2020; Rahman et al. 2021). Phytochemical analysis reveals that its leaves and bark contain several bioactive compounds, including terpenoids such as koetjapic acid, along with limonoids, flavonoids, and triterpenes. These compounds have been linked in other studies to pharmacological activities such as antioxidant, antimicrobial, and tissue-repairing effects, which favor wound healing (Bailly 2022). Despite these promising findings, no in vivo study had yet explored the wound-healing activity of *S. koetjape*.

Therefore, this study sought to assess the potential wound-healing activity of ethanolic leaf extract of *S. koetjape* using the excision wound model in Swiss albino mice, a well-established method for investigating wound repair. This model provided a reliable way to assess wound closure by measuring wound contraction rate over time (Criollo-Mendoza et al. 2023). The study not only validated traditional claims that *S. koetjape* leaves and bark are used in folk medicine as poultices for cuts and abrasions, decoctions for washing small open wounds, and hot compresses for soft-tissue recovery, but also contributed to the growing body of evidence on medicinal plants as affordable and sustainable wound-healing agents. This is particularly valuable for developing plant-based alternatives in regions with limited access to expensive synthetic wound-care products.

➤ Conceptual Framework

This study utilized this conceptual framework that focused on the woundhealing potential of *Sandoricum koetjape* ethanolic leaf extract through topical application of treatment.

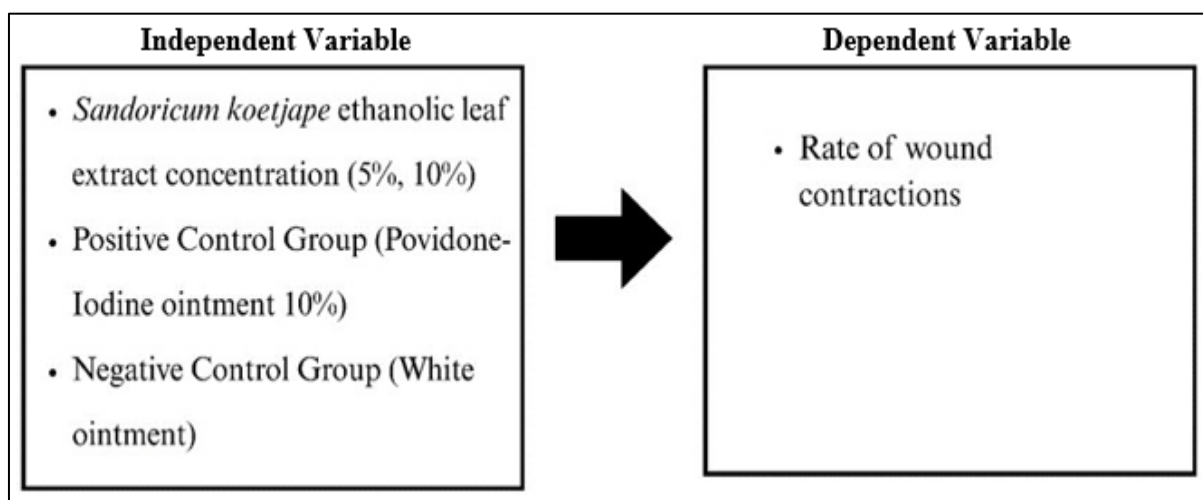


Fig 1 Conceptual Framework of the Study

Figure 1 above shows the conceptual framework on the effect of the independent variables, namely *S. koetjape* ethanolic leaf extract at varying concentrations (5% and 10%), the positive control (10% povidone-iodine ointment), and the negative control (white ointment), on the dependent variable, which is the rate of wound contraction. The concentration of 10% povidone-iodine was selected because it is the pharmacopeial standard formulation widely used in both clinical wound care and preclinical wound-healing studies, ensuring reliable comparison. These independent variables are expected to directly influence the rate of wound contraction, which serves as a key indicator of the wound-healing process. Wound contraction as the dependent variable is the key indicator of the wound-healing process, reflecting the reduction in wound size over time as a result of tissue regeneration and repair. This represents how different treatments were expected to influence the wound healing response in the test subjects. By assessing the wound contraction, this model aimed to determine the effectiveness of *S. koetjape* in promoting tissue repair compared to the standard wound treatment and the negative control.

➤ Research Objectives

This study aimed to assess the potential wound-healing effect of the ethanolic leaf extract of *Sandoricum koetjape* (Meliaceae) using an excision wound model in Swiss albino mice, contributing to the search for effective and natural wound treatment alternatives. Specifically, this study aimed to:

- Identify the percentage yield of *S. koetjape* ethanolic leaf extract.
- Confirm the phytochemical constituents present in *S. koetjape* ethanolic leaf extract.
- Determine the approximate toxic dose of *S. koetjape* ethanolic leaf extract through acute dermal toxicity testing in Swiss albino mice, following OECD Guideline 402 for Acute Dermal Toxicity.
- Formulate an ointment with the crude extract and prepare control ointments for positive and negative standards.
- Observe wound healing through assessment of the rate of contraction (Day 0, 3, 7, and 14). With corresponding key healing phases: baseline (Day 0), inflammation (Day 3), proliferation (Day 7), and remodeling (Day 14).
- Compare the wound healing rate of the extract-treated group with a standard treatment and a negative control group, then evaluate the extract's effectiveness in promoting wound contraction relative to these treatments.

➤ Research Hypothesis

This study evaluated the wound-healing effects of the ethanolic leaf extract of *S. koetjape*, formulated into topical ointments. The researchers sought to determine whether these formulations significantly enhanced wound healing compared to standard treatments.

Null Hypothesis (H_0): The application of *S. koetjape* ethanolic leaf extract has no wound-healing activity and shows no significant difference between its ethanolic leaf extract and positive control group (Povidone-Iodine ointment 10%) and the negative control group (White ointment) on dermal excision wounds in Swiss albino mice.

Alternative Hypothesis (H_1): The application of *S. koetjape* ethanolic leaf extract has wound-healing activity and shows a significant difference between its ethanol extract and the positive control group (Povidone-Iodine ointment 10%) and the negative control group (White ointment) on dermal excision wounds in Swiss albino mice.

➤ Significance of the Study

This study contributed significantly across different areas by investigating the potential wound-healing properties of the ethanolic leaf extract of *S. koetjape* in topical ointment formulations. The findings enhanced the understanding of natural remedies and offered practical benefits to the following involved parties:

- Healthcare Practitioners. The study provided alternative options for healthcare practitioners in the use of effective, plant-based wound care treatments. By demonstrating the possible benefits of *S. koetjape* extracts, the study offered guidance for healthcare practices and helped improve ways of caring for patients.
- Community. The findings of this research benefited local communities by promoting the use of *S. koetjape* in topical treatments. This may lead to greater reliance on locally available, plant-based resources, reducing dependence on costly commercial wound care products.
- Future Researchers. For researchers, this study expanded the body of knowledge on the efficacy of *S. koetjape* in wound care. It provided an overview for further studies on the therapeutic properties of *S. koetjape* and similar natural products, contributing to the development of new, research-based wound care solutions.
- Students. This study developed students' understanding of how plant-based extracts can be applied in modern therapeutic formulations. It served as a learning tool, especially for pharmacy students, emphasizing the role of natural products in creating effective and accessible treatments.

➤ Scope and Limitations

This study focused on assessing the potential wound-healing effect of the ethanolic leaf extract of *Sandoricum koetjape* (Meliaceae) using an excision wound model in Swiss albino mice. This model was selected as it enabled straightforward measurement of wound contraction rate, which served as a reliable indicator of cutaneous healing. The study will employed three comparative groups: a negative control group treated with white ointment, a positive control group treated with 10% povidone-iodine ointment, and test groups treated with 5% and 10% *S. koetjape* ethanolic leaf extract ointments. The experimental observation was carried out over a 14-day period, with measurements taken at day 0 (baseline), day 3 (inflammation phase), day 7 (proliferation phase), and day 14 (remodeling phase), corresponding to the main stages of wound healing in mice. The parameters observed included the rate of wound contraction. In addition, the approximate toxic dose of the extract was evaluated through acute dermal toxicity testing in accordance with OECD Guideline 402, ensuring the safety of the preparation prior to topical application.

The study did not extend to identifying or isolating individual compounds responsible for wound healing, nor did it evaluate other pharmacological activities of *S. koetjape* beyond its effect on wound repair. It also did not cover long-term toxicity, pharmacokinetics, or clinical application in humans, as these were beyond the capacity and timeframe of the current research. Results were therefore be interpreted only within the context of laboratory-based animal testing, specifically using Swiss albino mice.

➤ *Definition of Terms*

- *Sandoricum koetjape*. Refers to a tropical tree native to Southeast Asia, commonly known as Santol or Cotton fruit, whose ethanolic leaf extract was investigated for its potential wound-healing properties in an excision wound model.
- Excision Wound Model. Refers to the experimental procedure where a defined area of skin was surgically removed in Swiss albino mice to create a wound. In this study, it was used to evaluate how treatments, including *S. koetjape* ethanolic leaf extract, influenced wound contraction and healing.
- Phytochemical. Refers to natural chemical/s found in plants that can have health effects, such as flavonoids, tannins, terpenoids, and limonoids that contributed to tissue repair and wound healing.
- Povidone-Iodine. Refers to a standard antiseptic substance used to prevent infection and promote wound healing which served as the positive control group in the study.
- Ethanolic Extract. Refers to the preparation obtained by soaking the powdered leaves of *S. koetjape* in 90% ethanol to extract useful substances. It was formulated into topical ointments for wound-healing evaluation.
- Topical Ointment. Refers to a greasy, oil-based formulation applied to the wound area. Ointments containing *S. koetjape* extract, Povidone-Iodine, or a nonmedicated ointment base (White ointment) were used for comparison of healing effects.
- *OECD (Organization for Economic Co-operation and Development)*
Refers to the standardized protocol for assessing acute dermal toxicity. It served as the reference guideline for evaluating the safety of *S. koetjape* extract before wound application.
- ✓ Wound Healing. Refers to the body's way of fixing the skin and tissue after an injury. In this study, wound healing was specifically assessed through the rate of wound contraction over time in mice treated with different ointment formulations.
- ✓ Inflammation. The second phase of wound healing that is a protective biological response triggered by tissue injury or infection. It involved immune cell activation and increased blood flow to eliminate harmful stimuli and initiate healing.
- ✓ Wound Contraction. This refers to the reduction in wound size over time, which was measured as the drawing together of wound edges.
- ✓ Hemostasis. The initial phase of wound healing that stops bleeding through vasoconstriction and clot formation. It created a temporary matrix that supported subsequent tissue repair.
- ✓ Bioactive. A bioactive substance produces a measurable biological effect on living tissues. In this study, it refers to compounds that influenced cellular processes such as inflammation, proliferation, or regeneration.
- ✓ Proliferation. The third phase in wound healing during which cells, particularly fibroblasts and endothelial cells, rapidly divide and multiply to form new tissue. This process contributed to the reconstruction of the wound bed, including the formation of granulation tissue, blood vessels, and extracellular matrix.

CHAPTER TWO

REVIEW OF LITERATURE AND RELATED STUDIES

This chapter provided background information on the Potential Wound Healing Effect of Ethanolic Extract of *Sandoricum koetjape* (Meliaceae).

➤ *Sandoricum koetjape* (Meliaceae)

Sandoricum koetjape belongs to the family Meliaceae. It is a perennial tropical tree commonly known as Santol, Cotton Fruit, or Kechapi in various parts of the world. The plant is native to Maritime Southeast Asia and is widely cultivated in countries such as the Philippines, Indonesia, Malaysia, Thailand, Vietnam, and India, as well as in other tropical regions for its edible fruits and medicinal uses. Traditionally, its different parts particularly the leaves, bark, and fruits have been utilized in folk medicine for ailments such as fever, diarrhea, and skin infections. Recent phytochemical studies revealed that *S. koetjape* contains terpenoids, limonoids, flavonoids, saponins, and triterpenes, compounds associated with antioxidant, antimicrobial, and anti-inflammatory activities that contributed to its potential woundhealing effects (Wijaya 2022; Bailly 2022).

• *Sandoricum koetjape* (Meliaceae) Taxonomy

The Taxonomic classification of *Sandoricum koetjape* according to authoritative botanical databases such as the International Plant Names Index (IPNI 2024), Plants of the World Online (POWO 2024), and World Flora Online (WFO 2024) was as follows:

- ✓ Kingdom: Plantae
- ✓ Phylum: Tracheophyta
- ✓ Division: Magnoliophyta
- ✓ Class: Magnoliopsida
- ✓ Order: Sapindales
- ✓ Family: Meliaceae
- ✓ Genus: *Sandoricum*
- ✓ Species: *Sandoricum koetjape*

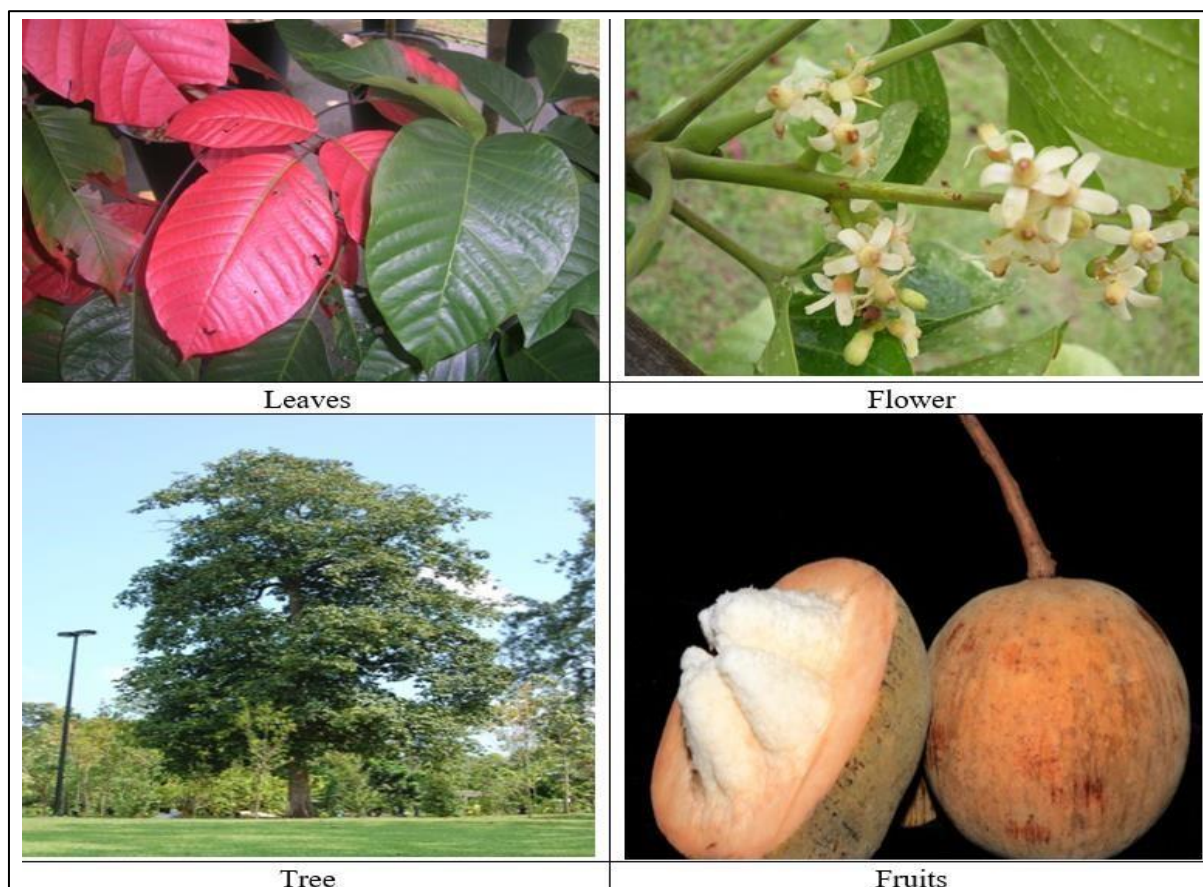


Fig 2 Photographs of the Leaves, Flowers, Tree, Fruit (the Flesh and Seeds of the Fruits) of *S. koetjape*.
Photo by National Parks Board (2025).

- *Sandoricum koetjape* (Meliaceae) Habitat

The *S. koetjape*, a tropical fruit tree that holds a significant place in Southeast Asian ecosystems. Known for its round, sweet-sour fruit, it has been valued both as food and for its ecological uses. Santol is native to the tropical forests of Southeast Asia, especially in countries like the Philippines, Malaysia, Thailand, and Indonesia. It thrives in warm, humid climates and grows best in regions that receive abundant rainfall. While it prefers tropical and subtropical regions, the Santol tree can also adapt to various soil types, provided there is good drainage. It requires full sunlight for good growth and fruit production. Santol is commonly seen in home gardens, village farms, and orchards, and it is sometimes found along roadsides and secondary forests. Aside from its fruits, it is valued for providing shade and as a source of wood (Forestry 2024).

- *Ethnobotanical Uses of Sandoricum koetjape* (Meliaceae)

Sandoricum koetjape is a plant used in traditional medicine in several parts of Southeast Asia, particularly in the Philippines, Indonesia, Malaysia, and Thailand. Agapin (2020) reported that in the Philippines, there is an abundance of *S. koetjape* where locals pound the leaves and bark into poultices applied on cuts, abrasions, and bruises to support the natural closure of wounds. In farming communities, decoctions prepared from the bark have been used as washes for small open wounds and sores when immediate medical care is not available, providing a practical household approach to first aid (Rahman et al. 2021). In some provinces, especially in rural Quezon, hot compresses made by boiling young santol leaves in water mixed with vinegar are applied to tender areas or after childbirth to help with recovery of minor soft-tissue injuries (Philippine Traditional Knowledge Digital Library 2025).

Such traditional applications of *S. koetjape* illustrated how the plant has been incorporated into local practices for managing minor injuries and maintaining skin integrity in community settings.

- *Plants from the Meliaceae Family with Wound-healing Properties*

The Meliaceae family, commonly referred to as the Mahogany family, is composed of tropical trees and shrubs known for their several diverse secondary metabolites. Table 1 below list several members of this family which were reported to exhibit pharmacological wound healing activities.

Table 1 Plant of Meliaceae Family of Wound Healing

Research Title	Members of Meliaceae family	Citations
Evaluation of wound healing and anti-inflammatory activities of 80% methanol crude extract and solvent fractions of <i>Trichilia dregeana</i> leaves in mice	<i>Trichilia dregeana</i>	Shewaye DG, Kahaliw W, Belete TM, Ahmed N. 2023. Evaluation of wound healing and anti-inflammatory activities of 80% methanol crude extract and solvent fractions of <i>Trichilia dregeana</i> (Meliaceae) leaves in mice. Evid Based Complement Alternat Med [Internet]. [cited 2025 Aug 27]; 9980866. Available from: doi: 10.1155/2023/9980866.
Phytochemical screening and wound healing activity of <i>Azadirachta indica</i> leaf extract in Wistar rats	<i>Azadirachta indica</i>	Akinmoladun FO, Komolafe TR, Komolafe OA, Farombi EO. 2021. Phytochemical screening and wound healing activity of <i>Azadirachta indica</i> leaf extract in Wistar rats. J Herbmed Pharmacol [Internet]. [cited 2025 Aug 31]; 10(2):166-172. Available from: doi:10.34172/jhp.2021.18
In-Vivo Therapeutic Evaluation of the Wound Healing Potential of Ethanolic Extract of <i>Trichilia heudelotii</i> Leaves in Wistar Rats	<i>Trichilia heudelotii</i>	Awolola GV, Jinadu TH, Yakubu MT, Oyewopo AO, Habibur RM. 2023. In-vivo therapeutic evaluation of the wound healing potential of ethanolic extract of <i>Trichilia heudelotii</i> leaves in Wistar rats [Internet]. [cited 2025 Sep 6]. Available from: https://www.researchgate.net/publication/376565698
Evaluating the antibacterial, antioxidant and wound healing properties of the stem bark extract of <i>Khaya grandifoliola</i> (Welw) CDC (Meliaceae) (African mahogany)	<i>Khaya grandifoliola</i>	Quartey AN, Orman E, Mireku-Gyimah NA, Goku PE, Alidu F, Boateng LO. 2020. Evaluating the antibacterial, antioxidant and wound healing properties of the stem bark extract of <i>Khaya grandifoliola</i> (Welw) cdc (Meliaceae) (African mahogany). Journal of Medicinal Plants Research [Internet]. [cited 08 Sept 2025];14(11):604–612. Available from: doi: https://doi.org/10.587/jmpr2020.7023 .

- *Sandoricum koetjape* (Meliaceae) Pharmacological Activities and Phytochemicals

S. koetjape, a member of the Meliaceae family, is widely recognized for its ethnomedicinal value and diverse phytochemical profile. Various parts of the plant, including its roots, bark, leaves, fruits, and seeds, contain bioactive compounds that contribute to its therapeutic potential (Bailly 2022). The roots contain limonoids, terpenoids, and tannins; the stem, bark, and heartwood are rich in cytotoxic terpenoids such as koetjapic acid and sentulic acid; the fruit pulp and hull possess flavonoids, phenolics, and nutritional constituents; and the seeds are abundant in limonoids and insecticidal triterpenoids such as sandoricin (Wijaya 2022). This broad phytochemical distribution underlay the plant's wide-ranging pharmacological activities, including antimicrobial, antiinflammatory, antioxidant, anticancer, and insecticidal effects.

According to the study by Wijaya (2022), the leaves of *S. koetjape* are particularly rich in limonoids, terpenoids, flavonoids, saponins, tannins, and phenolic acids. These metabolites provided a pharmacological basis for the plant's use against inflammatory and infectious conditions and were relevant to wound-healing processes (Hapid et al. 2024).

Among these phytochemicals, several classes showed properties related to wound healing. Terpenoids, including koetjapic and sentulic acids, exert antiinflammatory effects by downregulating nitric oxide synthase and proinflammatory cytokines, thereby preventing chronic inflammation that impairs healing (Armaghan et al. 2024). Limonoids, such as sandoripins, sandrapins, and sandoricin derivatives, demonstrate antimicrobial and immunomodulatory activities that help protect wounds from pathogenic infection while supporting immune balance. Flavonoids act as potent antioxidants, scavenging reactive oxygen species that cause oxidative damage, while also promoting keratinocyte migration and collagen synthesis essential for re-epithelialization (CedilloCortezano et al. 2024). Tannins, with their astringent and antimicrobial properties, facilitate wound contraction and form a protective barrier against microbial invasion. Saponins were shown to enhance angiogenesis and stimulate collagen deposition, thereby accelerating granulation tissue formation and wound closure (Cedillo-Cortezano et al. 2024).

- ✓ *Flavonoids*

Flavonoids are polyphenolic compounds abundantly found in *S. koetjape*, known for their antioxidant and anti-inflammatory properties. They play a vital role in neutralizing free radicals, which can damage tissues and slow down the healing process (Zhang et al. 2022). In wound healing, flavonoids helped by promoting collagen synthesis, stimulating angiogenesis (formation of new blood vessels), and regulating inflammatory responses, resulting in faster tissue regeneration and improved wound closure (Carvalho et al. 2021).

The leaves and fruits of *S. koetjape* have traditionally been used to reduce inflammation and treat skin ailments, which aligned with the modern understanding of flavonoids' role in skin repair and regeneration (Wijaya 2022). These compounds also helped improve the tensile strength of the healed tissue, making the new skin stronger and more resilient.

- ✓ *Terpenoids*

Terpenoids are 5-carbon compound isoprene with oxygen-containing functional groups isolated from several plants that demonstrate a wide range of activities such as antioxidant, anti-inflammatory and anti-microbial activity Furthermore, due to their excellent cytoprotective activity, terpenoids based products played a significant role in wound healing activity. (Morgan Babu et al. 2021).

- ✓ *Limonoids*

Limonoids are a class of highly oxygenated modified triterpenoids with a diverse range of biological activities. Although with restricted occurrence in the plant kingdom, these compounds are found extensively in the Meliaceae and Rutaceae families. Limonoids are of great interest in science given that the small number of plant families where they occur exhibit a broad range of medicinal properties that promote health and prevent disease (Olatunji et al. 2021).

Limonoids are primarily found in the Meliaceae and Rutaceae (citrus) families and occur less frequently in Simaroubaceae and Cenoraceae families (Odebunmi et al. 2021).

- ✓ *Phenolic Acids*

Phenolic acids are a type of phenolic compound with potent antioxidant and anti-inflammatory activities. These compounds neutralize free radicals and reactive oxygen species (ROS), protecting cells from oxidative damage during the early stages of wound healing (Zhang et al. 2022).

Phenolic acids also enhanced fibroblast proliferation and collagen synthesis, both critical for forming the extracellular matrix and strengthening newly regenerated tissue (Usman et al. 2024). Furthermore, they exhibited antimicrobial effects, preventing infections that could slow the healing process.

➤ Wound

• *Defining Wounds*

A wound generally refers to any injury that causes a disruption in the structure and function of living tissue (Bowers et al. 2020). Specifically, it is the breakdown of the skin or underlying soft tissue, which can result from trauma, burns, infections, or other pathological conditions (Nagle et al. 2023). This disruption exposes the tissue to microbial invasion and impairs normal healing processes, potentially leading to persistent inflammation, delayed repair, and complications if not properly managed (Uberoi et al. 2024). Wounds, therefore, were not only a structural compromise but also a functional challenge to tissue integrity, creating risks for infection and prolonged recovery (Park and De 2025).

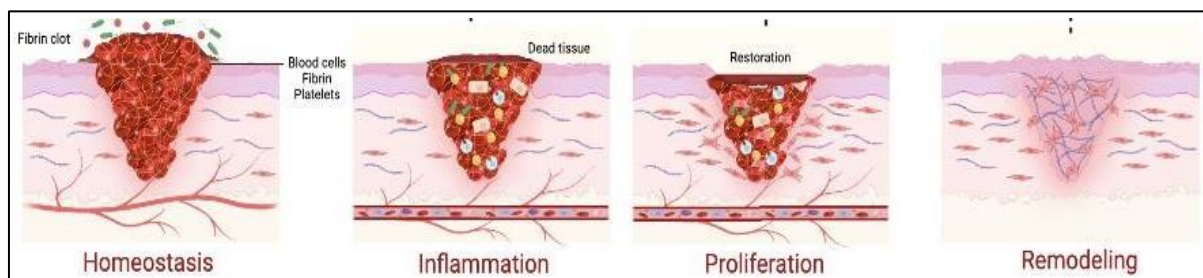
• *Wound Healing Physiology*

Fig 3 Phases of Wound Healing. Photo by Choudhary et al. (2024)

When the skin is injured, wound healing progressed through four phases: hemostasis, inflammation, proliferation, and remodeling. During hemostasis, the body quickly works to stop bleeding by forming a blood clot. Platelets stick to the injured area and become activated. These activated platelets attract more platelets, creating a platelet plug. At the same time, thrombin is produced through the body's coagulation pathways and converts fibrinogen into fibrin. The fibrin weaves through the platelet plug and trapped blood cells, forming a stable clot that seals the wound and prevents further bleeding (Grubbs and Manna 2023).

The second phase of wound healing, the inflammation phase, was essential for initiating tissue repair and preventing infection. This phase begins immediately after injury and typically lasts for several days. It is characterized by the infiltration of inflammatory cells, such as neutrophils and macrophages, into the wound site. These cells perform essential functions, including the clearance of pathogens and debris, and the release of cytokines that regulate subsequent healing processes (Gao et al. 2024). In the proliferation phase, the wound surface was restored through processes such as re-epithelialization, collagen production, extracellular matrix development, and re-establishment of the blood supply. During the final remodeling phase, regenerative activities decreased, allowing for the reorganization of connective tissue and initiation of the wound contraction response (Baron et al. 2020).

A wound was considered healed when the skin's barrier function and structural integrity were fully restored, indicating the completion of the wound healing process. This involved the orderly progression through the hemostasis, inflammation, proliferation, and remodeling phases. Key indicators of healing included stable epithelialization, absence of drainage, pain, or infection, which collectively demonstrated effective restoration of the tissue (Simon et al. 2024). The wound healing process is intricately linked to angiogenesis, the formation of new blood vessels and collagen remodeling. These mechanisms were vital for restoring the strength and functionality of the healed tissue. Effective healing outcomes were indicated by reduced scarring and improved tissue strength, which served as key criteria for determining whether a wound had fully healed (Mathew-Steiner et al. 2021).

Wounds are categorized into acute and chronic types. Acute wounds typically heal within about four weeks, following a predictable course and usually without significant complications. In contrast, chronic wounds are more challenging to treat because they heal slowly, persist for longer periods, and often progress irregularly, which can result in serious complications that may require tissue removal (Nguyen et al. 2023).

• *Causes of Wound*

Wound healing is a complex process influenced by multiple factors that can either support or slow down recovery. Infection was a major challenge in wound management, as disruptions in the skin microbiome can allow normally harmless bacteria to become pathogenic, thereby delaying healing (Yang et al. 2024). The skin normally hosts bacteria as part of its microbiota, but when their levels increase or the barrier is breached, these microbes can contribute to infection and impair wound healing (Uberoi et al. 2024).

Wounds may result from a variety of internal or external causes. Common external causes include abrasions, lacerations, puncture wounds, and gunshot injuries, each displaying distinct characteristics based on the injury type. Abrasions typically emerge when the skin is rubbed or scraped against a surface. Lacerations occur when blunt or sharp objects tear the skin. Puncture wounds result from pointed objects piercing the tissue, and gunshot wounds stem from bullets entering or passing through the body, often leading to extensive tissue injury (MSD Manual 2024; West Coast Wound Care 2023).

The common cause of wounds was injury, which can range from superficial damage to deep wounds that require surgery. Burns are another type of wound, typically caused by direct or indirect contact with heat, electricity, radiation, or chemical agents, and in severe cases, may lead to cell death requiring hospitalization. Burns are classified by their depth. First-degree burns involve only the skin's outer layer, causing pain, redness, and swelling. Second-degree burns extend to both the outer and underlying layers, leading to pain, redness, swelling, and blistering. Third-degree burns affect the deepest layers of skin and sometimes underlying tissues, resulting in white or blackened, burned skin that may be numb (MedlinePlus 2024). In addition, prolonged pressure on certain areas of the body, especially over bony parts like the heels, ankles, hips, and tailbone, can reduce blood flow to the skin and tissues. This lack of circulation can lead to pressure ulcers, also known as bedsores or pressure injuries. These sores may start as mild skin damage but can worsen over time, reaching deeper layers of tissue and becoming infected if not properly cared for, which could make healing more difficult and slow recovery (Nguyen et al. 2023).

➤ *Antiseptic: Povidone Iodine*

Povidone iodine was one of the most established antiseptics used in wound care. It is a compound made of polyvinylpyrrolidone and iodine, which slowly releases free iodine to destroy bacteria, fungi, viruses, and even spores by disrupting proteins, lipids, and nucleic acids in microbial cells. When used at appropriate concentrations, it was observed to also stimulate granulation tissue formation, and encourage reepithelialization, leading to faster wound closure (Girgis et al. 2023; Chelms-Burlacu et al. 2023). Widely used for years in wound care, povidone iodine served as a broadspectrum antiseptic irrigation solution and promoted faster wound contraction (Durani et al. 2024).

• *The Excision Wound Model*

The Excision Wound Model was a widely used experimental method for studying wound healing and evaluating the effectiveness of therapeutic agents. It involved the surgical creation of a full-thickness wound, usually around 1.5 cm in diameter, on the dorsal surface of laboratory animals anesthetized usually using EMLA cream (topical mixture of lidocaine and prilocaine). Once the wound was created, the animals were divided into treatment groups based on the study's objectives. The healing process was then monitored using the key parameters such as the epithelialization period, which referred to the number of days required for the wound to completely close without residual scarring, and wound contraction, which was measured by tracing the wound area over time and calculating the percentage reduction in wound size (Choudhary et al. 2021). Excision wounds closely resemble acute clinical wounds and primarily heal by second intention, where the wound edges are not sutured but heal naturally through granulation and tissue regeneration. This procedure involved removing all skin layers, including the epidermis, dermis, and subcutaneous tissue, making it ideal for studying the natural wound healing process. The model was especially valuable for analyzing the sequential stages of healing, such as inflammation, granulation tissue formation, angiogenesis, re-epithelialization, and tissue remodeling. Wound closure was assessed by comparing the wound size at different time intervals to its original measurement, providing reliable and quantitative data on the progression of healing (Goswami et al. 2022).

➤ *Theoretical Framework Feedback*

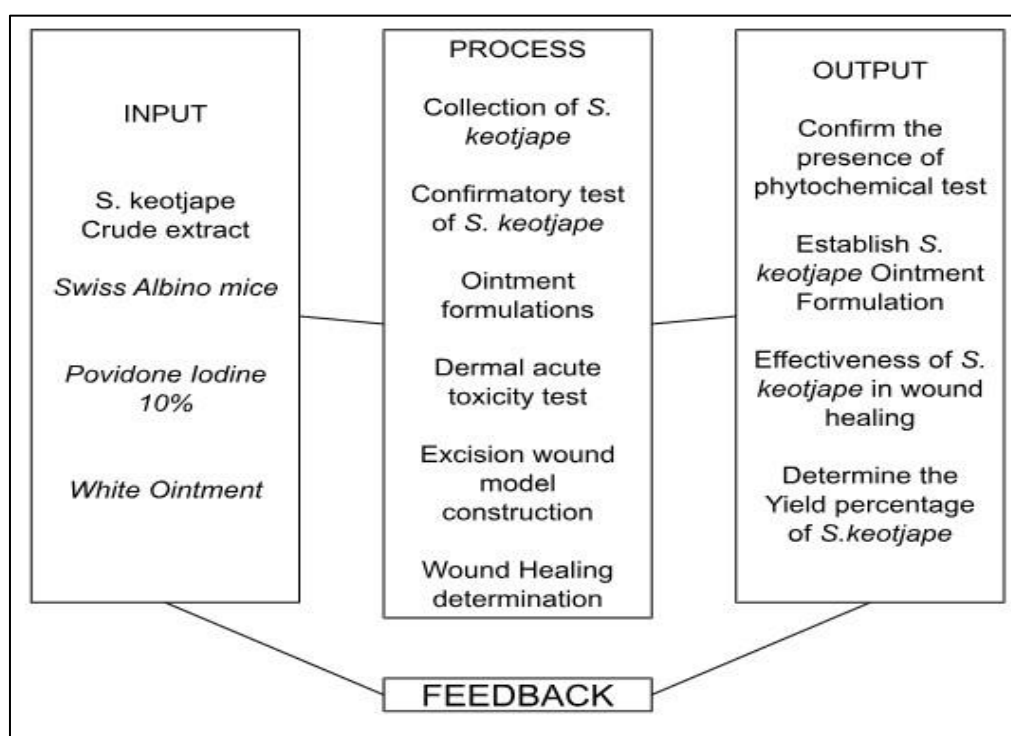


Fig 4 Theoretical Framework

This theoretical framework provided a comprehensive understanding of the wound healing properties of *S. koetjape*. The study adopted an “input-process-output” framework to explain the relationship between its elements. The inputs included the formulated ointment from *S. koetjape* ethanolic leaf extract, the Swiss albino mice serving as experimental models, and the use of 10% povidone-iodine ointment as the positive control. The process involved the collection and preparation of *S. koetjape* leaves, phytochemical screening ointment formulation, dermal acute toxicity testing, and excision wound model construction, and wound-healing evaluation. The outputs were established formulation of *S. koetjape* ointment and the determination of its effectiveness in promoting wound contraction. This framework offers a systematic approach to evaluating the wound-healing potential of *S. koetjape*.

➤ Related Studies

The table below (Table 2) presented a summary of key literature that supported the current study, focusing on extraction techniques, wound healing agents, and the biological potential of *Sandoricum koetjape*. It included studies that described different extraction methods such as maceration, percolation, and infusion for medicinal and aromatic plants; the established safety and multifunctional properties of petroleum jelly; and the wound healing efficacy of povidone-iodine. Furthermore, it highlighted studies exploring the antibacterial, angiogenic, and pharmacological activities of *S. koetjape*.

Table 2 Related Studies on Extraction Methods and Wound Healing Agents

Study Title	Key Findings	Citations
Maceration, Percolation and Infusion Techniques of Extraction of Medicinal and Aromatic Plants	Maceration was identified as a reliable, cost-effective method for extracting bioactive compounds like flavonoids and tannins without damaging heat-sensitive compounds, making it ideal for plant-based wound healing formulations.	Philadelphia University. 2024. Comparative overview of maceration and percolation processes for plant-based extractions. [Internet]. [cited 2025 Sep 26]; Available from: https://www.philadelphia.edu.jo/academics/s_telfah/uploads/method%20of%20extraction.pdf
Petroleum Jelly: A Comprehensive Review of Its History, Uses, and Safety	Petroleum jelly was utilized as a negative control in wound healing experiments due to its inert nature and barrier-forming properties, allowing accurate comparison of treatments.	Kamrani P, Hedrick J, Marks JG, Zaenglein AL. 2024. Petroleum jelly: A comprehensive review of its history, uses, and safety. <i>J Am Acad Dermatol</i> . Available from: https://www.sciencedirect.com/science/article/abs/pii/S0190962223011076
Povidone-Iodine in Wound Healing and Prevention of Wound Infections	Povidone-iodine served as a standard positive control in wound healing studies because of its broad-spectrum antimicrobial action and ability to accelerate granulation tissue formation.	Gmur MK, Karpiński TM. 2020. Povidone-iodine in wound healing and prevention of wound infections. <i>Eur J Biol Res</i> . Available from: http://www.journals.tmkarpinski.com/index.php/ejbr/article/view/325/148
A Topical Ointment Formulation Containing Leaves' Powder of <i>Lawsonia inermis</i> Accelerates Excision Wound Healing in Wistar Rats	Herbal ointment containing <i>Lawsonia inermis</i> leaf powder significantly promoted excision wound healing compared to control groups, demonstrating the value of natural plant-based wound treatments.	Yassine KA, Houari H, Mokhtar B, Karim A, Hadjer S, Bediaf I. 2020. A topical ointment formulation containing leaves' powder of <i>Lawsonia inermis</i> accelerate excision wound healing in Wistar rats. <i>J Ethnopharmacol</i> [Internet]. [cited 2025 Sep 26]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7429373/
Antibacterial Activity of <i>Sandoricum koetjape</i> Leaf Ethanolic Extract	Ethanolic leaf extract of <i>S. koetjape</i> inhibited the growth of <i>Staphylococcus aureus</i> and <i>E. coli</i> , demonstrating its ability to prevent wound infections that could delay healing.	Santos MG, et al. 2020. Antibacterial activity of <i>Sandoricum koetjape</i> leaf ethanolic extract. <i>DLSMHSI J Res</i> [Internet]. [cited 2025 Sep 26]; Available from: https://greenprints.dlshsi.edu.ph/grade_12/104
Angiogenic Modulatory Activity of <i>Sandoricum koetjape</i> Ethanolic Leaf Extract	Using a duck embryo CAM assay, ethanolic extract of <i>S. koetjape</i> demonstrated angiogenesis modulation, indicating its potential role in the proliferative phase of wound healing.	Fajri R, et al. 2021. Angiogenic modulatory activity of <i>Sandoricum koetjape</i> ethanolic leaf extract observed through duck embryo CAM assay. <i>Biomed Pharmacol J</i> [Internet]. [cited 2025 Sep 26]; Available from: https://www.biomedsciresearch.com

Ethnomedicinal, Phytochemicals, and Pharmacological Aspects of Sentul (<i>Sandoricum koetjape</i>)	Reviewed the traditional medicinal uses and identified bioactive compounds such as triterpenes, limonoids, and flavonoids in <i>S. koetjape</i> . Reported pharmacological activities included antioxidant, antibacterial, antifungal, and antiinflammatory properties.	Alvian D, Susilo RJ, Pratiwi D. 2021. Ethnomedicinal, phytochemicals, and pharmacological aspects of sentul (<i>Sandoricum koetjape</i>). <i>Biomedich J Sci Tech Res</i> [Internet]. [cited 2025 Sep 26]; Available from: https://sciencebiology.org/index.php/biomedich/article/view/177
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CHAPTER THREE

METHODOLOGY

This chapter discusses the procedures that were used in exploring the extract of *S. koetjape* 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 methods of analyzing the outcome.

➤ Materials

This study required different materials, including plant samples, laboratory chemicals, equipment, and experimental animals. The main material used was the leaves of *Sandoricum koetjape* (Santol), which served as the source of the ethanolic extract tested for its potential wound healing effects. The experiment was conducted in the Department of Pharmacy Laboratory at Adventist Medical Center College, Barangay San Miguel, Iligan City, Philippines, which was equipped with the necessary tools and facilities for plant extraction, animal handling, and data analysis. Various chemicals and solvents, such as ethanol, were used for the extraction and preparation of treatments. Standard laboratory instruments like beakers, flasks, surgical scissors, forceps, and digital calipers were utilized for precise measurement and handling during the experiment. In addition, healthy Swiss Albino mice were used as the test subjects to evaluate the effect of the prepared extract on wound healing. These materials were essential to ensure the proper conduct of the study and the accuracy of its results.

• Plant Materials

Fresh and mature Santol leaves were collected from healthy trees growing in Lambaguhon, Barangay San Roque, Iligan City, Lanao del Norte, Philippines (8.2578° N, 124.2618° E). Care was taken to select leaves that were free from visible signs of disease, insect damage, or physical defects to ensure the quality and reliability of the extract. Once harvested, the leaves were placed in clean, breathable bags or containers to prevent moisture buildup and were transported immediately to the laboratory for further processing.

Upon arrival at the laboratory, the collected plant samples underwent identification and verification by a licensed botanist from Mindanao State University–Iligan Institute of Technology (MSU-IIT), Tibanga, Iligan City. This process ensured that the plant was correctly identified as *S. koetjape*, eliminating the possibility of misidentification. After identification, the leaves were washed thoroughly with clean running water to remove dirt, dust, and other contaminants that could interfere with the extraction process.

The clean leaves were air-dried under shade at room temperature (20–25°C) for 7–14 days, avoiding direct sunlight to prevent the degradation of heat- and light-sensitive compounds essential for their medicinal properties. The drying process continued until a reduction in the leaves' weight was observed, indicating the loss of excess moisture. Once dried, the leaves were pulverized into a fine powder using a Hanabishi Blender (Model HJB-326) and passed through mesh sieve no. 120. The powdered samples were then stored in airtight, properly labeled containers and kept in a cool, dry place until they were ready to be used for extraction (Krakowska-Sieprawska et al. 2022).

• Standard Reagent and Chemicals

All solvents, chemicals, and reagents used in this study were of analytical and laboratory grade. They were obtained from the Department of Pharmacy Laboratory at Adventist Medical Center College. This ensured that all chemicals met high-quality standards and supported the reliability and reproducibility of the research.

Distilled water was used throughout the study for preparing solutions, washing glassware, and diluting reagents. Filtration of the plant extract was carried out using filter paper to separate the liquid extract from coarse plant material. All solvents and reagents were obtained from a reputable supplier to ensure pharmaceutical-grade quality and to minimize errors caused by impurities or degraded chemicals. Following standard laboratory practices for sourcing chemicals ensured consistency and reliability of results.

For the wound healing test, normal saline solution (0.9% NaCl) was used for wound irrigation and cleansing of the excision wound area in mice prior to treatment to prevent contamination and infection. A 10% povidone-iodine ointment was used as the positive control, as it is a standard antiseptic for normal wound healing due to its well-documented ability to promote tissue regeneration and prevent infection (Monstrey et al. 2023). On the other hand, white ointment was used as the negative control, serving as an inert base that does not possess wound healing activity. Its purpose was to provide a comparison for the ethanolic leaf extract of *S. koetjape*, ensuring that any observed effects on wound contraction and healing were due to the bioactive compounds present in the extract and not the ointment base itself. The use of both positive and negative controls was essential, as it allowed for accurate evaluation of the extract's wound healing potential and ensured the reliability of the experimental results.

• Instruments and Apparatus

The instruments and apparatus required for this study were prepared and set up in the pharmaceutical laboratory of Adventist Medical Center College, Department of Pharmacy, which provided an accessible and well-equipped environment for conducting

experimental research. These tools played a crucial role in the accurate preparation of the ethanolic leaf extract of *Sandoricum koetjape* and in ensuring the precision and reliability of the wound-healing experiment.

A Hanabishi Blender (Model HJB-326) was used to pulverize the fully dried santol leaves into fine powder to increase the material's surface area for extraction. The resulting powder was then passed through a mesh sieve No. 120 to obtain uniform particle size. For extraction, 90% ethanol served as the solvent in a maceration apparatus, selected for its suitability in extracting heat-sensitive phytochemicals at room temperature.

A rotary evaporator was employed afterward to remove excess ethanol under reduced pressure, ensuring low-temperature solvent evaporation and preservation of extract integrity. The concentrated extract was collected, weighed, and stored in airtight, properly labeled containers in a cool and dry environment until needed for ointment preparation and wound-healing experiments.

➤ Research Flow

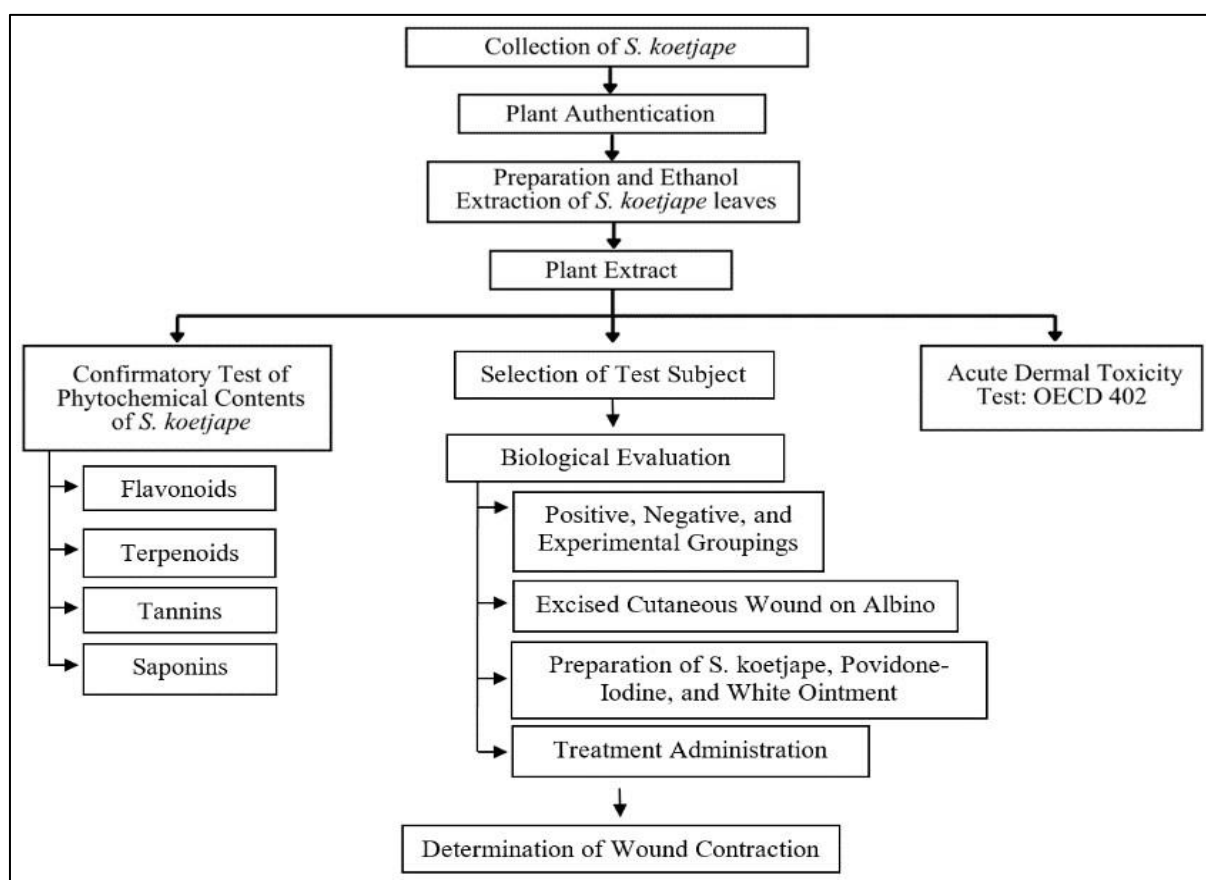


Fig 5 Flowchart of the Study Process

The figure above (Figure 5) illustrated the step-by-step process of the study, from plant collection to data analysis. It began with the collection and authentication of *Sandoricum koetjape* leaves, followed by cleaning, shade-drying, grinding, and sieving to prepare the material for extraction. The powdered leaves underwent ethanol maceration, filtration, and rotary evaporation to yield the crude extract.

This extract was then used for two phases of analysis. The first involved phytochemical screening to verify the presence of compounds such as flavonoids, terpenoids, tannins, and saponins. Second, an acute dermal toxicity test was performed following the OECD 402 guidelines to evaluate the safety of the extract prior to biological testing.

Following these analyses, ointments containing varying concentrations of the extract were prepared, along with the positive control (Povidone-Iodine) and negative control (White Ointment). These preparations were applied in the biological evaluation phase, which involved the selection of test animals, their grouping into control and experimental sets, and the creation of excision wounds on Albino Mice. The formulated treatments were then administered, and the rate of wound contraction was measured and recorded over the study period to assess the wound healing potential of *S. koetjape* leaf extract.

➤ Extraction and Characterization of Plant

The samples of *Sandoricum koetjape* were separated from dirt or foreign materials and then cleaned with running water until clean. They were then spread out to dry at room temperature in a shaded area. Drying continued for about one to two weeks, or until the leaves showed a constant weight, indicating that excess moisture had been removed. After this, the dried leaves were ground into powder and passed through a fine mesh sieve (no. 120 sieve) to obtain uniform particles. The powdered material was then stored in sealed, labeled containers in a cool, dry place until it was used for ethanol extraction.

• Extraction of *S. koetjape*

The extraction was carried out by the maceration method using ethanol as the solvent. A total of 200 grams of *S. koetjape* powder, placed in a stoppered 1000 mL Erlenmeyer flask, was macerated using ethanol at a ratio of 1:10 (Wutsqa et al. 2021). Soaking was done for 72 hours with regular agitation using an orbital shaker set at a speed of 50 rpm to ensure efficient extraction of the bioactive components. After 72 hours, the extract was filtered using filter paper to obtain the liquid extract. The separation of the crude extract from the alcohol solvent resulted in a concentrated extract (Mathews 2024). This process was carried out using a water bath under reduced pressure at a temperature of $\leq 60^{\circ}\text{C}$. When approximately 90% of the solvent had been removed, the crude extract was collected and stored under proper conditions, preferably in a cool and dry place, to prevent chemical degradation.

The concentrated extract was weighed, and the percentage yield was determined in relation to the weight of the plant material initially used. The average percentage yield of the crude ethanolic extract was determined by using the formula given below:

$$\% (w/w) \text{ yield} = \frac{\text{weight of crude extract (g)}}{\text{weight of dried leaf sample (g)}} \times 100$$

• Confirmatory Test of *Sandoricum koetjape* (Meliaceae)

Confirmatory tests were carried out to verify the results of the phytochemical screening, as ensuring reliable detection of plant constituents was important for the subsequent assessment of their biological properties (Chelmuş-Burlacu et al. 2023). The confirmatory tests were conducted at the Department of Pharmacy, Adventist Medical Center College. The researchers performed the confirmatory tests following standard methods.

The table 2 below outlined the confirmatory tests for the major classes of phytochemicals present in *S. koetjape* leaf extract. These confirmatory tests are essential for validating the presence of bioactive compounds such as terpenoids, flavonoids, saponins, and tannins, which contributed to the plant's pharmacological properties.

Table 3 Confirmatory Test of *S. koetjape* (Meliaceae) Leaf Extract.

Phytochemical	Test	Procedure	Positive Result	References
Terpenoids	Liebermann-Burchard Test	Cool the dried crude extract and defat with hexane. Add into the extract with 3-5mL of Ferric chloride reagent and filter. Divide the filtrate into two equal parts. Add into the first part 10 mL of dichloromethane and stir. Pipette out the lower dichloromethane extract and dry the extract by passing it through 100 mg anhydrous sulfate. Filter and treat the filtrate with 3 drops of acetic anhydride then 1 drop of concentrated sulfuric acid (Wutsqa et al. 2021)	Blue to green, red, or violet.	Wutsqa YU, Suratman S, Sari LA. 2021. Detection of terpenoids and steroids in <i>Lindsaea obtusa</i> with thin layer chromatography. Asian J Nat Prod Biochem [Internet]. [cited 2025 Sept 25]; 19(2):66-69. Available from: doi: 10.13057/biofar/ f190204.
Tannins	Ferric chloride Test	Evaporate the crude extract to almost dryness under a boiling water bath. Add 3-5 drops of 5% Ferric chloride and the second part serves as a control (Dubale et al. 2023)	Blue or greenblack color of precipitate .	Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S. 2023. Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in ethiopia. J Exp Pharmacol [Internet]. [cited 2025 Sept 25];15:51-62. Available from: doi: 10.2147/JEP.S37 9805.

Saponins	Froth Test	Take 2g of plant extract and transfer it into a test tube. Add 10 ml of distilled water and put the stopper on the test tube. Shake the test tube vigorously for 30 seconds. Allow the test tube to stand for 10 mins then observe for a “honeycomb” froth (Saadah et al. 2022)	Froth is 2 cm in height.	Saadah S, Tulandi SM, Rohman RA. 2022. Phytochemical and gas chromatographymass spectrometry profiling of two plant parts of <i>Sandoricum koetjape</i> . Biodiversitas [Internet]. [cited 2025 Sept 25]; 23(12):61996207. Available from: doi:10.13057/bio div/d231214.
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➤ Selection of Animal Species

Adult male Swiss Albino mice were chosen for this study because males typically exhibit more stable hormone levels compared to females, which improves consistency and reduces variability in wound healing outcomes (Alrouji et al. 2023). The mice were 8–12 weeks old, weighing around 20–30 grams. Only those with healthy, uninjured skin were included to ensure that baseline dermal status did not influence the responses in toxicity and healing assessments.

The animals were obtained from the animal facility of AMCC College of Pharmacy, Iligan City, Philippines, which maintains standard environmental conditions. Before beginning any procedures, the mice underwent an acclimatization period in the housing facility to adapt to laboratory conditions and reduce stress.

• Ethical Considerations and IACUC Approval

Before starting the experiment, the researchers applied for approval from the Institutional Animal Care and Use Committee (IACUC) of AMCC College of Pharmacy, Iligan City, Philippines. This step ensured that all parts of the study involving the use of mice, including handling, wound creation, and treatment application, followed proper ethical guidelines. The research proposal was reviewed to confirm that it complied with the OECD (2017) guidelines for the care and use of laboratory animals.

During the experiment, every effort was made to handle the animals responsibly and minimize any pain or discomfort. Anesthesia and clean techniques were used when creating the wounds, and the mice were carefully monitored daily for any signs of stress or illness. They were housed in clean cages with proper ventilation, provided with sufficient food and water, and maintained under standard laboratory conditions.

The actual procedures began only after the IACUC granted official approval. This ensured that the study respected animal welfare and followed both institutional and ethical standards throughout the research process.

• Housing and Feeding condition

The animals were housed individually in clean, well-ventilated transparent plastic containers with lids (capacity: 5000 mL; dimensions approximately 22.9 cm × 15.2 cm × 12.7 cm), originally designed for multipurpose storage. Each mouse was placed in a separate container to ensure isolation and prevent contact with other mice.

The containers were modified to allow proper ventilation and contained sterilized wood shavings as bedding, which were regularly replaced to maintain hygiene. The animals were kept under standardized conditions, including a controlled room temperature of 22–24 °C, a relative humidity of 50% ± 10%, and a 12-hour light/dark cycle. The mice had ad libitum access to a nutritionally balanced pellet diet formulated for laboratory mice, and filtered drinking water was provided in sterilized bottles.

Before the start of the experiment, the mice were acclimatized to these housing and feeding conditions for a minimum of 7 days. During this period, daily monitoring was conducted to ensure the health and welfare of the animals, including observation for any signs of stress, illness, or abnormal behavior.

➤ OECD 402: Acute Dermal Toxicity

The OECD 402 test was used to assess the potential toxic effects of a substance when applied to the skin. Prior to the wound healing study, the methanolic leaf extract of *Sandoricum koetjape* underwent acute dermal toxicity testing following a modified version of the 2017 OECD 402 guidelines. An initial dose was selected to produce observable toxicity without causing severe harm. Subsequent doses were then adjusted to determine the level that resulted in either toxicity with at most one death, no toxic effects at the highest dose, or mortality at the lowest dose.

• Administration of Doses

For the dermal toxicity assessment, the ethanolic leaf extract of *S. koetjape* was topically applied to the shaved dorsal skin of Swiss Albino mice in accordance with OECD Guideline No. 402 for Acute Dermal Toxicity. The ethanolic leaf extract was administered at sequential dose levels of 50 mg/kg, 200 mg/kg, 1000 mg/kg, and 2000 mg/kg body weight, following the stepwise dosing scheme recommended by OECD Guideline No. 402 (2017). These doses were administered to determine any potential dose-dependent toxic effects. Single animals were used in a sequential manner with two animals used at any selected dose level in the main study.

The test preparations were uniformly applied over the designated area of intact skin and gently massaged to ensure full coverage. A thin layer of gauze was placed over the application site and secured with non-irritating tape to prevent accidental removal or ingestion. To further minimize the risk of licking or grooming, each mouse was fitted with an Elizabethan collar (e-collar) during the 24-hour exposure period. After 24 hours of exposure, the dressing was removed, and the treated area was rinsed with lukewarm water to eliminate any residual material.

The mice were then observed daily for 14 consecutive days to monitor signs of local irritation, inflammation, or systemic toxicity. Clinical observations, including changes in behavior, posture, body weight, respiration, and coat condition, were recorded following a scoring system adapted from OECD 402, where 0 indicated normal and 3 indicated severe manifestation.

These observations helped identify any potential dermal or systemic reactions associated with the topical application of the extract.

➤ Formulation

The test formulations were prepared to evaluate the wound healing activity of *S. koetjape* (santol) leaf extract. Various concentrations of the extract were incorporated into an ointment base, while povidone-iodine ointment served as the positive control and white base ointment as the negative control. Each formulation was carefully prepared following standard pharmaceutical compounding procedures to ensure consistency, stability, and comparability across treatment groups.

• Preparation of Ointments from the *S. koetjape* Extract

Two ointment formulations containing *S. koetjape* ethanolic leaf extract were prepared at concentrations of 5% and 10% (w/w). This concentration range was commonly used for topical plant-extract ointments in excision wound models and allowed comparison of a lower and higher dose to detect any doseresponse effect. A USP-based white ointment composed of petroleum jelly and white wax served as the ointment base, in accordance with pharmacopeial standards for semisolid preparations that utilize inert excipients to ensure stability and compatibility (Bao et al. 2020).

The following tables (Tables 4 and 5) presented the formulation details for the 5% and 10% *S. koetjape* ethanolic leaf extract ointments, respectively.

Each formulation used white ointment as the base to provide an occlusive, stable, and inert medium for topical application. White wax was included to improve consistency and structural integrity, while petroleum jelly functioned as the primary occlusive vehicle. The ethanolic leaf extract served as the active component responsible for the potential wound-healing activity.

Table 4 5% *S. koetjape* Ethanolic Leaf Extract Ointment Preparation

Ingredients	Concentration (% w/w)	Quantity
<i>S. koetjape</i> Ethanolic Leaf Extract	5%	5g
Petroleum jelly	90%	90g
White Wax	5%	5g
	Total:	100g

Table 5 10% *S. koetjape* Ethanolic Leaf Extract Ointment Preparation

Ingredients	Concentration (% w/w)	Quantity
<i>S. koetjape</i> Ethanolic Leaf Extract	10%	10g
Petroleum jelly	85%	85g
White Wax	5%	5g
	Total:	100g

The preparation began by gently melting the white ointment base in a water bath at around 60°C until it liquefied. The measured amount of *S. koetjape* extract (corresponding to 5% or 10% of the total weight) was then incorporated gradually with continuous stirring to ensure even distribution. Mixing was continued until a homogeneous consistency was achieved.

The finished mixture was poured into clean, sterile containers, labeled accordingly, and left to cool at room temperature until it solidified. The ointments were stored in tightly sealed jars under cool, dry conditions until they were used for the wound-healing activity tests in Swiss Albino mice.

- *Preparation of Positive Control (Povidone - Iodine Ointment)*

The positive control was prepared in the form of a 10% povidone-iodine ointment. The “10%” designation meant there were 10 g of povidone-iodine per 100 g of total ointment, resulting in a final formulation containing 10% w/w active ingredient.

The table below (Table 6) presented the formulation details of the 10% povidone-iodine ointment, which was prepared following a modified formulation adapted from the formulary of Wuhan Diao Pharmaceutical Co. Ltd (2023). The selection of povidone-iodine as the positive control was based on its well-documented efficacy in wound management, particularly in preventing infection and promoting granulation tissue formation during the inflammatory phase of wound healing. The table outlined the specific ingredients used, their respective concentrations, and the quantities required to produce a 100 g batch of ointment.

Table 6 Ingredients and Quantity of the 10% Povidone-Iodine Ointment

Ingredients	Concentration (% w/w)	Quantity
Povidone Iodine	10%	10g
Potassium Iodate	0.3%	0.3g
PEG-400	25%	25g
PEG-4000	25%	25g
Xylitol	10%	10g
Glycerol	17%	17g
Sodium Lauryl sulfate	1%	1g
Purified water	11.7%	11.7mL
Total:	100g	

In this preparation, polyethylene glycol-400 and PEG-4000 were heated at 78–82°C for 30 minutes with thorough stirring. Xylitol was then added to the mixture and stirred for 15 minutes at the same temperature. Separately, sodium lauryl sulfate was dissolved in purified water while stirring for 15 minutes. The mixtures from both containers were then blended and stirred for another 15 minutes.

Upon cooling to 60–65°C, potassium iodate and povidone-iodine powder were incorporated through continuous stirring, resulting in a consistent and homogeneous ointment. The final ointment was cooled and stored in an amber-colored ointment jar for use in the study (Wuhan Diao Pharmaceutical Co. Ltd. 2023). The final preparation yielded a stable 10% povidone-iodine ointment suitable for topical application and was stored under controlled conditions until further use in the experiment (Saadah et al. 2022).

- *Preparation of Negative Control (White Ointment)*

The negative control was prepared using a simple ointment base without any active antimicrobial compound. This formulation consisted solely of white petroleum jelly, which served as an inert vehicle for ointment preparations. It provided a smooth and occlusive base that helped maintain moisture at the wound site but had no therapeutic or antimicrobial activity. In wound management research, such a formulation was commonly used as a negative control to differentiate the natural healing process from the effects produced by active treatments.

In this study, the ointment was prepared by accurately weighing 95 g of white petroleum jelly, melting it over a water bath, and stirring continuously to ensure uniformity. The molten mixture was then cooled to room temperature and stored in sterile containers until use. This preparation served as the baseline comparison for evaluating the wound healing activity of the plant extract formulations (Puri et al. 2021).

The table below (Table 7) presented the composition of the negative control, which consisted of a plain white ointment as the base. This formulation did not contain any active ingredient and was used to establish a baseline for comparison. The base was composed of white wax and petroleum jelly, providing a smooth, semi-solid consistency with occlusive properties that helped retain moisture and protect the wound area. By serving as a neutral vehicle, the white ointment allowed the evaluation of wound healing effects to be attributed specifically to the test and positive control formulations.

Table 7 Ingredients and Quantity of the White Ointment

Ingredients	Concentration (% w/w)	Quantity
White Wax	5%	5g
Petroleum jelly	95%	95g
Total:		100g

➤ Evaluation

The wound-healing potential of *Sandoricum koetjape* extract was evaluated using an excision wound model in Swiss Albino mice, with wound contraction serving as the primary parameter. The rate of contraction was monitored at specific time intervals to compare healing progress among the different treatment groups, including the control and extract-based formulations. This evaluation helped determine the effectiveness of the *S. koetjape* extract in enhancing wound closure compared to standard and untreated controls.

• Experimental Groupings

The animals used to determine the potential wound-healing activity of *S. koetjape* were adult albino mice weighing 20–30 grams, which were the preferred strain. Twenty-four experimental mice were divided into four groups, with six mice in each group.

Group 1, the positive control, was treated topically with povidone-iodine ointment. Group 2, the negative control, was treated topically with white ointment. Group 3 was treated topically with *S. koetjape* ethanolic leaf extract 5% in ointment form. Group 4 was treated topically with *S. koetjape* ethanolic leaf extract 10% in ointment form.

Each mouse received a daily topical treatment of 0.1 mL of the assigned ointment. This dosage was administered consistently throughout the study period to ensure uniform exposure across all experimental groups.

Table 8 Experimental Design for Wound-Healing Study of *S. koetjape* (Meliaceae)

Group Label	Treatment Group	# of Animals
Group 1	Positive control: 10% Povidone-iodine ointment	6
Group 2	Negative control: White ointment	6
Group 3	<i>S. koetjape</i> ethanolic leaf extract (ointment) 5%	6
Group 4	<i>S. koetjape</i> ethanolic leaf extract (ointment) 10%	6

• Excision Wound Model

A mouse excisional wound model was employed, as it is a well-established method that closely simulates acute clinical wounds and has been widely used in studies investigating wound-healing mechanisms (Hora et al. 2024). Prior to wounding, the mice were anesthetized using EMLA cream (lidocaine + prilocaine 5%, 5 g) and exposed for at least 60 minutes to ensure a painless procedure (Krohn et al. 2021).

The dorsal fur was carefully shaved using a sterilized blade, and the surgical area was disinfected with 70% ethanol. To create standardized wounds, the dorsal skin was gently lifted to form a fold, and a sterile 6-mm biopsy punch was used to excise a circular section of full-thickness skin. Each animal received one wound on the dorsal area, which was treated with the ointment formulation containing the plant extract at a fixed concentration. This ensured consistent dosing across all animals.

After application, an Elizabethan collar was placed on each mouse for 24 hours to prevent self-licking or scratching, ensuring proper absorption and controlled exposure. The animals were then transferred to the animal facility for observation during recovery from anesthesia and subsequently returned to their individual cages once fully conscious.

➤ Data Collection

The data collected throughout this study were systematically recorded. Wound healing progress of the experimental animals was monitored by measuring the wound area at different intervals throughout the study period. Wounds were measured immediately after wound creation (Day 0) and on specific observation days (Day 3, Day 7, and Day 14) using digital Vernier calipers. The data were organized to evaluate the wound-healing potential of *Sandoricum koetjape* (Santol) ethanolic leaf extract in ointment formulations at varying concentrations.

Each measurement was recorded individually for each mouse. Rows represented individual subjects, while columns indicated the days of measurement and the corresponding wound sizes. All wounds were labeled according to their assigned treatment groups, including the negative control (white ointment), positive control (10% povidone-iodine ointment), and the different concentrations of *S. koetjape* extract (5% and 10%), ensuring clear differentiation between groups. All collected data were systematically organized to track healing progress and served as the basis for statistical evaluation.

• Wound Healing Progress and Area Reduction Analysis

Photographs of each wound were taken to provide visual documentation of the healing process.

The wound area was estimated using the ellipse approximation formula:

$$\text{Area} = \pi \times \frac{L}{2} \times \frac{W}{2}$$

Where L is the longest diameter and W is the diameter perpendicular to it. The percentage of wound contraction was then calculated using the following formula:

$$\% \text{ Contraction} = \frac{Area_0 - Area_n}{Area_0} \times 100$$

Where Area 0 represents the initial wound area (Day 0) and Area n represents the wound area at a given time point during the observation period. This calculation allow for a quantitative assessment of wound closure over time.

➤ Data Analysis

The data obtained from the excision wound healing experiment were analyzed using one-way analysis of variance (one-way ANOVA) to determine whether there were statistically significant differences among the treatment groups. The independent variable in this analysis was the type of treatment, which included the negative control (ointment base), positive control (povidone-iodine), and varying concentrations of the ethanolic leaf extract of *Sandoricum koetjape*. The dependent variable was the wound area or percentage of wound contraction, representing the progression of wound healing.

The data were expressed as mean ± standard deviation (SD). One-way ANOVA was performed separately for each observation day (Day 0, Day 3, Day 7, and Day 14) to compare the effects of the different treatments at specific time points. When significant differences were detected, a post-hoc test was conducted to identify which specific groups differed from each other.

A p-value of less than 0.05 was considered statistically significant, indicating that the observed differences were unlikely due to chance. This statistical approach allowed for the comparison of treatment effects on wound healing and helped determine the efficacy of *S. koetjape* extract relative to the control groups.

CHAPTER FOUR

RESULTS AND DISCUSSION

This chapter presents the results observed and analyzed from the ethanolic leaf extract of *Sandoricum koetjape* (Meliaceae), including its descriptive analysis, extraction yield, phytochemical screening, and acute dermal toxicity assessment. It also discusses the formulation of ointments and their use in evaluating wound-healing activity using the excision wound model in Swiss Albino mice. The findings are based on wound contraction rates and comparisons between positive control group (PovidoneIodine ointment 10%) and the negative group (White ointment), with corresponding discussions and interpretations.

➤ Preparation of Plant Extract

The crude ethanolic leaf extract of *Sandoricum koetjape* (Meliaceae) were evaluated, and the observations are presented in Table 9.



Fig 6 The Crude Ethanolic Leaf Extract of *S. koetjape* (Meliaceae)

Table 9 Evaluation of the Crude Ethanolic Leaf Extract of *Sandoricum koetjape* (Meliaceae)

Parameter	Observation
Color	Dark greenish-brown
Odor	Distinct herbal odor
Consistency	Viscous, semi-solid
Residue Formation	Dense residue
Container Adhesion	Dried components adhered to surface

• Percentage Yield

The extraction process resulted in an average percentage yield of 14.57%, based on a triplicate analysis of using a 20-gram sample. The table 10 below presents the individual yield obtained from each trial.

Table 10 Percentage Yield of the Ethanolic Leaf Extract of *S. koetjape* (Meliaceae)

Trial	Weight of Dried Leaves	Weight of Crude Extract	Percentage Yield
1	20 g	3 g	15%
2	20 g	2.64 g	13.2%
3	20 g	3.10 g	15.5%
		MEAN VALUE	14.57%

➤ Confirmatory Test

The phytochemical screening was conducted at the Adventist Medical Center College Pharmacy Laboratory in Iligan City to identify the bioactive components within the crude ethanolic leaf extract of *S. koetjape*. The analysis confirmed the presence of several key plant compounds, specifically flavonoids, tannins, saponins, and terpenoids. These constituents are widely recognized for their pharmacological roles in accelerating tissue repair and managing inflammation. The presence of these secondary metabolites suggests a strong biochemical basis for the wound-healing properties observed in the Santol leaf extract.

Table 11 Phytochemical Confirmation Test of the Ethanolic leaf Extract of *S. koetjape* (Meliaceae)

Plant Constituents	Tests	Visible Results	Interpretation	
Flavonoids	Shinoda's Test		Orange Coloration	Positive
Tannins	Ferric chloride Test		Greenish - brown Coloration	Positive
Saponins	Froth Test		Froth is 2 cm in height	Positive
Terpenoids	Liebermann-Burchard Test		Pink-red Coloration	Positive

➤ Isolation of Mice

During the isolation period, the mice were individually housed in containers measuring approximately 22.9 cm × 15.2 cm × 12.7 cm, which were modified to allow proper ventilation. Sterilized wood shavings were used as bedding material and were replaced every other day, together with regular cage cleaning, to maintain hygienic conditions throughout the study.

The animals were maintained under standardized environmental conditions, including a controlled room temperature of 22–24°C, relative humidity of 50% ± 10%, and a 12-hour light/dark cycle. All mice were provided with ad libitum access to a nutritionally balanced pellet diet and distilled drinking water, with approximately 30 g of pellets and 60 mL of water allocated per animal and replenished as needed.

Throughout the 7-day acclimatization period, the mice were regularly monitored prior to the experiment. Body weight measurements obtained using a calibrated weighing scale showed a gradual increase over time, indicating normal growth. Behavioral observations revealed no signs of distress or abnormal activity, as all mice remained active and exhibited normal behavior during the study period. These findings suggest that the housing and environmental conditions were appropriate and did not adversely affect the health status of the animals.

➤ Acute Dermal Toxicity - Fixed Dose Procedure

The acute dermal toxicity of the test substance was evaluated using a fixed dose procedure at six dose levels: 50, 200, 1000, 2000, and 5000 mg/kg body weight. This initial phase served as the dose range-finding study, following OECD Guideline 402, where each dose level was tested on one mouse. Dosing started at 50 mg/kg and was increased step by step to higher doses. Each animal was observed for 14 days for any signs of toxicity.

During the observation period, the animals were monitored for clinical symptoms such as changes in behavior, skin reactions, tremors, convulsions, and other abnormal responses. No mortality or signs of toxicity were observed at any of the tested dose levels. Even at the highest dose of 5000 mg/kg, which corresponds to the 5% and 10% plant extract ointment formulations, there were no signs of skin irritation, toxicity, or abnormal behavior. All animals remained normal in appearance and activity throughout the study. The results of the dose range-finding study are summarized in Table 12.

Table 12 Summary of Dose Range-Finding Results and Selected Dose for Main Study

Dose (mg/kg BW)	Number of Animals	Observed Toxicity	Mortality	Remarks
50	1	None	0	No adverse effect
200	1	None	0	No adverse effect
1000	1	None	0	No adverse effect
2000	1	None	0	Selected for main study
5000	1	None	0	No adverse effect

Since no death or toxic effects were observed during the dose range-finding phase, the study proceeded to the main study. In this phase, a dose of 2000 mg/kg was applied, and two additional mice were used, as recommended in OECD Guideline 402.

The animals were again observed for 14 days for signs of toxicity or mortality. Throughout the observation period, no mortality was recorded in any treatment group.

Furthermore, all treated animals exhibited no observable signs of toxicity, suggesting that the test substance was well tolerated at all administered doses under the conditions of the study and that the lethal dose (LD50) of this extract in mice is likely higher than 2000 mg/kg.

In addition, a gross necropsy examination was carried out at the end of the observation period to assess any potential internal effects of the test substance. The liver, spleen, and kidneys were examined, including measuring their weights (liver, spleen, right and left kidneys) and the length of the spleen. The organs were also observed for any visible lesions or unusual changes. As summarized in Table 13 below, all organs appeared normal in terms of size, color, and overall appearance, and no lesions or abnormalities were found. These results suggest that the test substance did not cause any noticeable harmful effects on the internal organs following dermal exposure (refer to Appendix for detailed gross necropsy results).

Table 13 Summary of Gross Necropsy Findings Following Acute Dermal Toxicity Study

Parameter	Mean $\pm$ SD	Interpretation
Liver weight (g)	1.83 $\pm$ 0.34	Normal
Spleen weight (g)	0.11 $\pm$ 0.05	Normal
Spleen length (mm)	16.05 $\pm$ 3.17	Normal
Right kidney (g)	0.26 $\pm$ 0.05	Normal
Left kidney (g)	0.27 $\pm$ 0.05	Normal
Lesions observed	None	No abnormalities

➤ Formulation of Ointments

In the formulation of the ointments, petroleum jelly was used as the primary base component due to its semi-solid consistency, stability, and occlusive properties, which are beneficial in maintaining moisture and promoting wound healing. Petroleum jelly serves as the primary ointment base, adhering to recommended practices in pharmacopeial semisolid formulations, which emphasize the use of inert excipients to ensure stability and compatibility (Bao et al. 2020).

The finished product of 5% *S. koetjape* ointment appeared light to medium brown with a uniform mixture and a slight herbaceous scent. The 10% formulation showed a deeper brown color with a more noticeable herbaceous odor, reflecting its higher extract content. Both ointments had semi-solid consistencies and appeared wellmixed after the preparation.

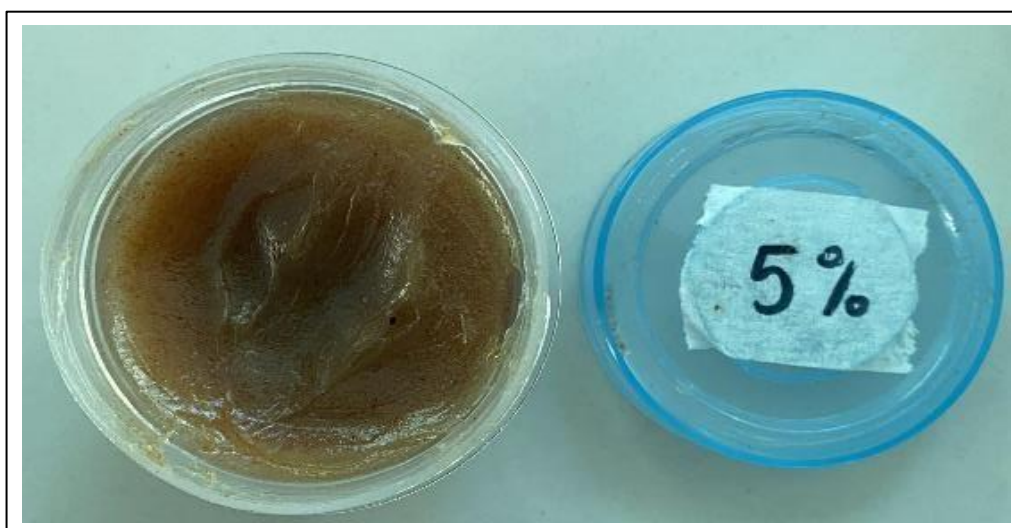


Fig 7 Finished Product of 5% *S.koetjape* Leaf Extract Ointment



Fig 8 Finished Product of 10% *S. koetjape* Leaf Extract Ointment

Meanwhile, the negative control ointment appeared as a smooth, white, semisolid preparation with a uniform consistency. It exhibited a stable, occlusive texture characteristic of a hydrophobic ointment base.



Fig 9 Finished Product of White Ointment

Following this, the povidone-iodine ointment preparation appeared as a brown, semi-solid preparation with a smooth and uniform consistency. It exhibited a characteristic iodine odor and a stable, homogeneous texture, indicating proper incorporation of the active ingredient within the hydrophobic ointment base.



Fig 10 Finished Product of 10% Povidone-Iodine Ointment

➤ Wound Area Reduction Across the Observation Period

Wound area was examined to describe the visible reduction in wound size across the 14-day observation period. Because all mice began with the same Day 0 wound area of 28.27mm², the baseline measurement functioned as a common starting point rather than as a meaningful between-group comparison. The main descriptive interest was therefore the extent to which the wound area decreased from Day 0 to Day 14 within each treatment group.

Table 14 Descriptive Statistics for Wound Area by Treatment Group and Observation Days

Group	Day	<i>n</i>	<i>M</i>	<i>SD</i>	<i>SEM</i>	Min	Max
Negative Control Ointment	Day 0	6	28.27	0.00	0.00	28.27	28.27
Negative Control Ointment	Day 3	6	22.27	1.86	0.76	20.38	24.74
Negative Control Ointment	Day 7	6	10.56	2.08	0.85	8.10	13.12
Negative Control Ointment	Day 14	6	0.61	0.81	0.33	0.08	2.13
Positive Control Ointment	Day 0	6	28.27	0.00	0.00	28.27	28.27
Positive Control Ointment	Day 3	6	22.20	1.53	0.62	20.40	24.98
Positive Control Ointment	Day 7	6	10.51	2.30	0.94	8.30	13.84
Positive Control Ointment	Day 14	6	0.55	0.66	0.27	0.00	1.43
5% Concentration Ointment	Day 0	6	28.27	0.00	0.00	28.27	28.27
5% Concentration Ointment	Day 3	6	21.17	2.18	0.89	18.63	23.97
5% Concentration Ointment	Day 7	6	11.14	1.94	0.79	9.21	14.67
5% Concentration Ointment	Day 14	6	0.07	0.14	0.06	0.00	0.35
10% Concentration Ointment	Day 0	6	28.27	0.00	0.00	28.27	28.27
10% Concentration Ointment	Day 3	6	20.62	1.85	0.76	17.87	23.54
10% Concentration Ointment	Day 7	6	10.23	1.07	0.44	8.66	11.88
10% Concentration Ointment	Day 14	6	0.00	0.00	0.00	0.00	0.00

- Note. *M*= mean; *SD*= standard deviation; *SEM*= standard error of mean. Descriptive analysis were computed from the individual mouse-level wound area measurements. Lower wound-area value indicate smaller remaining wound size.

Table 14 shows that mean wound area decreased steadily across the observational period in all treatment groups. By Day 3, the mean wound area had decreased to 22.27 mm² in the negative group, 22.20 mm² in the positive group, 21.17mm² in 5% extract group, and 20.62mm² in the 10% extract group. By the Day 7, the corresponding mean wound areas were reduced further to 10.56 mm², 10.51 mm², 11.14 mm², and 10.23 mm², respectively. These values indicate that visible wound closure progressed substantially between Day 0 and Day 7 across all groups. By Day 14, the remaining mean wound areas were very small in all groups. The negative control group had a mean wound area of 0.61 mm², the positive control group had a mean wound area of 0.55 mm², the 5% extract group had a mean wound area of 0.07 mm², and the 10% extract group reached a mean wound area of 0.00 mm².

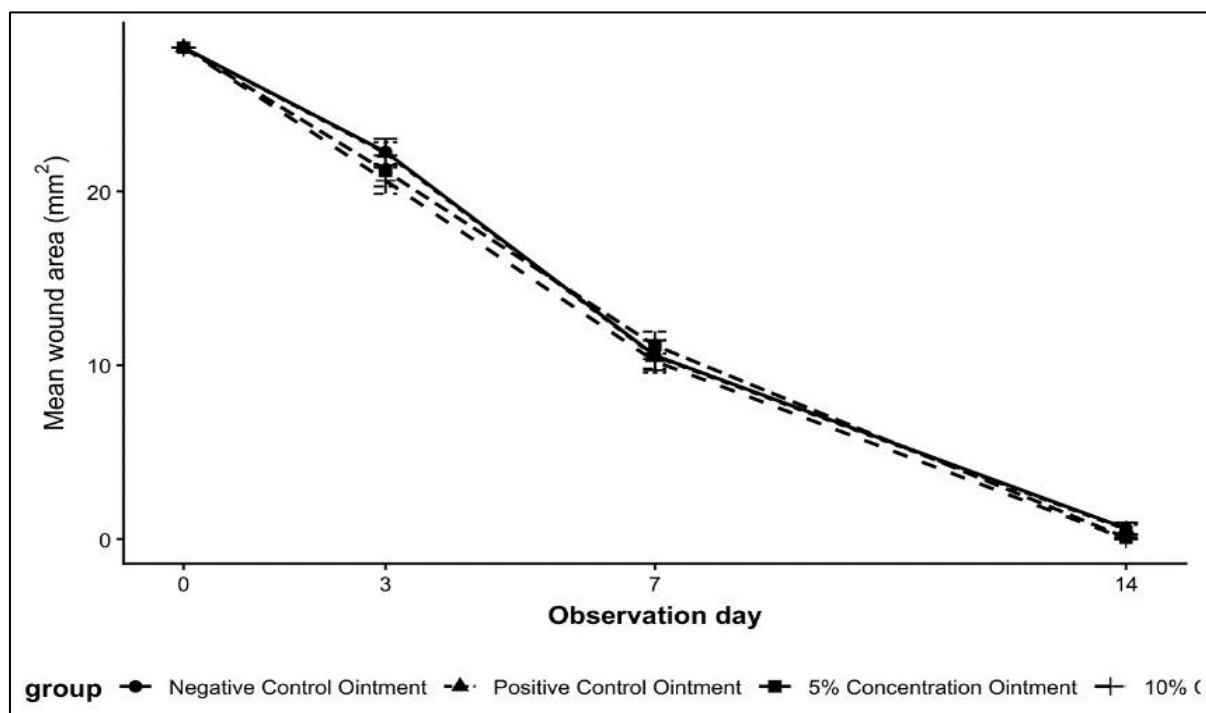


Fig 11 Mean Wound Area Over Time by Treatment Group. Error Bars Represent Standard Errors of the Mean.

Figure 11 visually supports this pattern, showing a consistent downward trajectory in wound area from Day 0 to Day 14 for each treatment group. The extract-treated groups showed very low residual wound areas by the final observation day, with the 10% extract group reaching complete mean closure based on the wound-area measurements. These descriptive wound-area findings support visible wound closure, but they should not be interpreted alone as proof of complete tissue restoration because wound area does not directly measure histological repair, collagen deposition, tensile strength, or epithelialization quality.

➤ Wound Contraction Progress Across Days

Wound contraction was examined as the primary indicator of visible wound closure because it expresses the percentage reduction in wound area relative to the Day 0 baseline. Higher contraction percentages indicate greater wound closure, with 100% representing complete visible contraction based on the recorded wound-area measurements. Although Day 0 was not a measured contraction outcome, it is shown descriptively as an implied 0% base-line to clarify the progression of contraction from the initial wound measurement to the succeeding observation days.

Table 15 Descriptive Statistics for Wound Contraction by Treatment Group and Observation Day.

Group	Day	n	M	SD	SEM	Min	Max
Negative Control Ointment	Day 0	6	0.00	0.00	0.00	0.00	0.00
Negative Control Ointment	Day 3	6	21.24	6.57	2.68	12.50	27.92
Negative Control Ointment	Day 7	6	62.64	7.36	3.00	53.61	71.37
Negative Control Ointment	Day 14	6	97.85	2.85	1.16	92.45	99.71
Positive Control Ointment	Day 0	6	0.00	0.00	0.00	0.00	0.00
Positive Control Ointment	Day 3	6	21.48	5.40	2.21	11.67	27.86
Positive Control Ointment	Day 7	6	62.83	8.12	3.31	51.06	70.66
Positive Control Ointment	Day 14	6	98.05	2.34	0.96	94.94	100.00
5% Concentration Ointment	Day 0	6	0.00	0.00	0.00	0.00	0.00
5% Concentration Ointment	Day 3	6	25.12	7.69	3.14	15.21	34.13
5% Concentration Ointment	Day 7	6	60.59	6.86	2.80	48.13	67.43
5% Concentration Ointment	Day 14	6	99.75	0.50	0.20	98.75	100.00
10% Concentration Ointment	Day 0	6	0.00	0.00	0.00	0.00	0.00
10% Concentration Ointment	Day 3	6	27.08	6.55	2.67	16.74	36.81
10% Concentration Ointment	Day 7	6	63.83	3.77	1.54	58.00	69.38
10% Concentration Ointment	Day 14	6	100.00	0.00	0.00	100.00	100.00

- *Note.* *M* = mean; *SD* = standard deviation; *SEM* = standard error of the mean. Day 0 is shown as an implied 0% wound-contraction baseline for descriptive completeness. Higher wound-contraction percentages indicate greater visible wound closure.

Table 15 shows that mean wound contraction increased markedly across the observation period in all treatment groups. On Day 3, the mean wound contraction was 21.24% in the negative control group, 21.48% in the positive control group, 25.12% in the 5% extract group, and 27.08% in the 10% extract group. By Day 7, contraction had increased to 62.64%, 62.83%, 60.59%, and 63.83%, respectively. This indicates that the largest visible increase in contraction occurred between the early and middle observation points, with all groups showing substantial wound closure by Day 7. By Day 14, all treatment groups had reached very high mean wound contraction. The negative control group reached 97.85%, the positive control group reached 98.05%, the 5% extract group reached 99.75%, and the 10% extract group reached 100.00%. Figure 12 visually supports this trend, showing steep upward trajectories in mean wound contraction across the observation period. The extract-treated groups, particularly the 10% extract group, showed the highest final-stage mean contraction. These descriptive results indicate progressive visible wound closure in all groups, with the extract-treated groups reaching near-complete or complete mean contraction by Day 14. The very high Day 14 values also indicate a ceiling effect, so later group comparisons should be interpreted with attention to the small practical differences that can occur near 100% contraction.

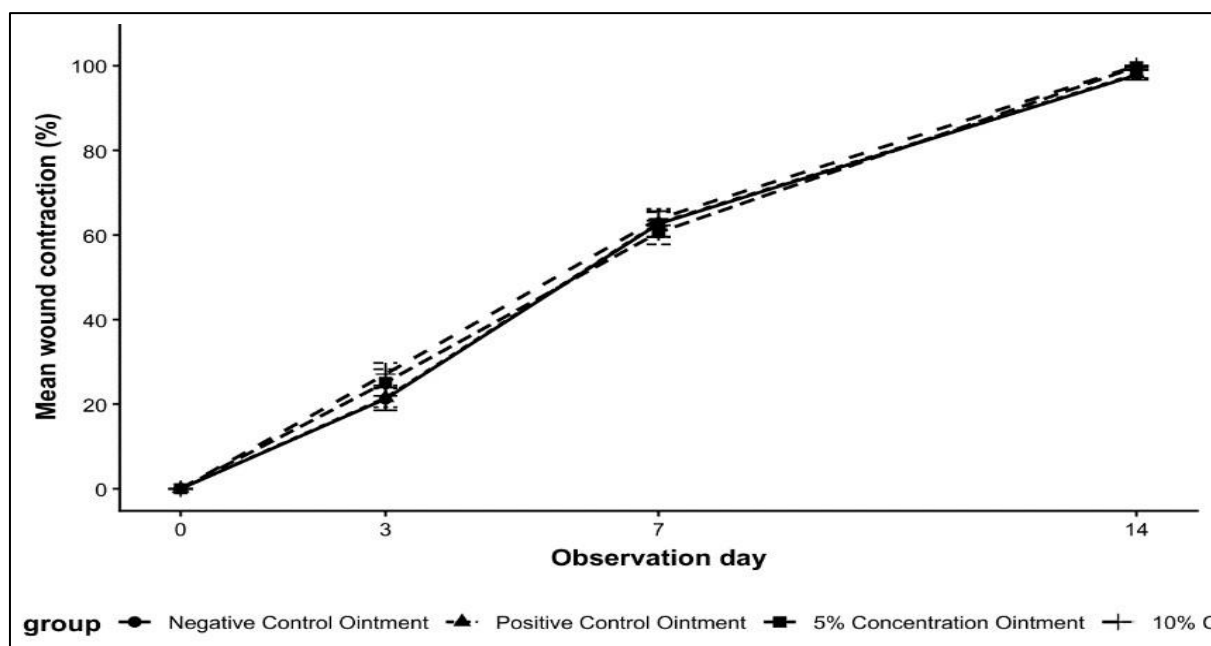


Fig 12 Mean Wound Contraction Over Time by Treatment Group. Day 0 is Shown as an Implied 0% Contraction Baseline. Error Bars Represent Standard Errors of the Mean.

➤ Overall Treatment-by-Day Pattern in Wound Contraction

To evaluate whether wound contraction differed by treatment group across the observation period, wound contraction percentages from Day 3, Day 7, and Day 14 were analyzed using a linear mixed-effects model. The model included fixed effects for treatment group, observation day, and the treatment group by day interaction, with a mouse-specific random intercept to account for repeated measurements from the same animal. The treatment group effect indicates whether average contraction differed across the four treatment conditions, the day effect indicates whether contraction changed over time, and the interaction effect indicates whether the pattern of contraction across days differed among treatment groups.

Table 16 Overall Treatment-by-Day Analysis of Wound Contraction.

Effect	df1	df2	F	p	Partial η^2	Interpretation
Treatment group	3	20	0.699	0.564	0.095	No statistically significant overall group effect
Day	2	40	1615.145	< .001	0.988	Wound contraction changed significantly over time
Treatment group x Day	6	40	0.767	0.600	0.103	No statistically significant differential healing trajectory by group

- *Note.* Wound contraction at Days 3, 7, and 14 was analyzed using a linear mixed-effects model with fixed effects for treatment group, observation day, and their interaction, and a mouse-specific random intercept. Partial η^2 is reported as an ANOVA-style effectsize summary for the fixed-effect tests.

Table 16 shows that the effect of day was statistically significant, $F(2, 40) = 1615.145$, $p < .001$, with a very large ANOVA-style effect-size estimate, partial $\eta^2 = 0.988$. This result indicates that wound contraction changed substantially across the observation period. In contrast, the overall treatment-group effect was not statistically significant, $F(3, 20) = 0.699$, $p = 0.564$, partial $\eta^2 = 0.095$, indicating that the average wound contraction across the measured days did not differ significantly among the four treatment groups. The treatment group by day interaction was also not statistically significant, $F(6, 40) = 0.767$, $p = 0.600$, partial $\eta^2 = 0.103$, indicating that the pattern of change in wound contraction over time was not statistically different across groups concentration ointment; 10% = 10% concentration ointment. Error bars represent 95% confidence intervals from a linear mixed effects model with mouse-specific random intercepts.

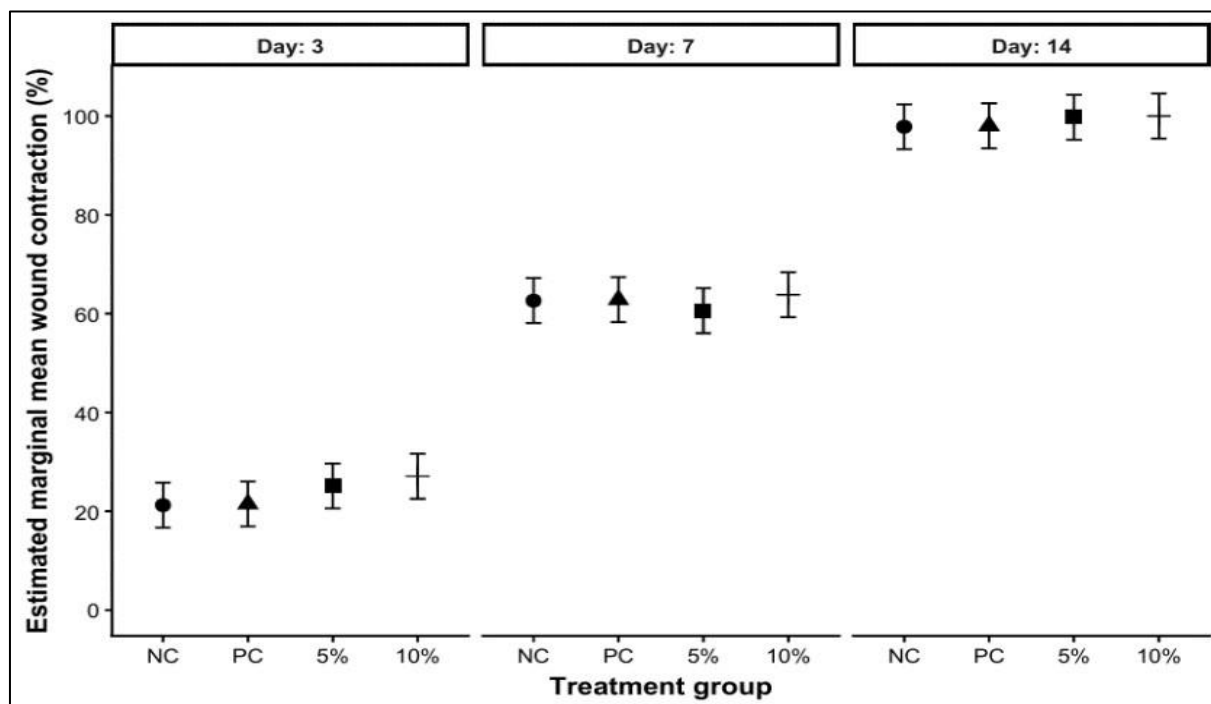


Fig 13 Estimated Marginal Means of Wound Contraction by Treatment Group Label and Day. NC = Negative Control Ointment; PC = Positive Control Ointment; 5% = 5% concentration ointment; 10% = 10% concentration ointment.

Figure 13 provides the model-based visualization of this result using estimated marginal means and 95% confidence intervals. The figure shows that all groups increased in wound contraction across the observation period, which is consistent with the significant main effect of day. Although the extract-treated groups, particularly the 10% extract group, showed numerically high contraction by Day 14, the confidence intervals overlapped across several group-by-day comparisons. Thus, the mixed-effects model supports a strong time-related wound-closure pattern but does not provide sufficient evidence that the extract-treated groups followed a statistically distinct healing trajectory from the control groups across Days 3, 7, and 14.

Accordingly, the main evidence from the overall model is temporal improvement rather than broad treatment-group separation. Day-specific analyses were therefore used to determine whether any group differences emerged at particular observation points.

➤ Day-Specific Group Comparisons

To further examine whether group differences emerged at specific observation points, separate day-specific comparisons of wound contraction were conducted for Day 3, Day 7, and Day 14. This analysis complements the overall mixed-effects model by identifying whether the treatment groups differed at individual stages of the observation period. Parametric one-way ANOVA was used for Days 3 and 7 because the diagnostic results supported its use, while the Kruskal–Wallis test was used for Day 14 because the contraction values showed a strong ceiling effect near complete closure.

Table 17 Day-Specific Comparison of Wound Contraction Among Treatment Groups

Day	Test used	Statistic	df	p	Effect size	Effect size type	Interpretation
Day 3	One-way ANOVA	1.120	3, 20	0.365	0.144	η^2	Not statistically significant group difference
Day 7	One-way ANOVA	0.244	3, 20	0.865	0.035	η^2	Not statistically significant group difference
Day 14	KruskalWallis	12.204	3	0.007	0.460	ϵ^2	Significant group difference

- *Note.* η^2 = eta-squared; ϵ^2 =epsilon-squared.

Table 17 shows that wound contraction did not differ significantly among treatment groups on Day 3, $F(3,20) = 1.120$, $p = 0.365$, $\eta^2 = 0.144$. Similarly, no statistically significant group difference was observed on Day 7, $F(3,20) = 0.244$, $p = 0.865$, $\eta^2 = 0.035$. These findings indicate that the treatment groups did not show clear statistical separation during the earlier and middle observation points of the woundhealing period.

By Day 14, the omnibus group comparison was statistically significant, $H(3) = 12.204$, $p = 0.007$, $\epsilon^2 = 0.460$. This result indicates that final wound contraction differed among at least some of the treatment groups. Figure 13 supports this interpretation by showing that the Day 14 group means were clustered near complete contraction, with the extract-treated groups having the highest final mean values.

The significant Day 14 result should be interpreted cautiously because most groups had already reached very high contraction by the final observation day. In this context, the omnibus finding reflects final-stage differences in visible closure near the upper boundary of the scale rather than broad evidence of superior biological wound repair. Post hoc comparisons were therefore examined to identify which specific group contrasts accounted for the Day 14 difference.

➤ *Post Hoc Comparisons at Day 14*

Because the Day 14 omnibus Kruskal–Wallis test indicated a statistically significant group difference, post hoc pairwise comparisons were conducted to identify which treatment groups differed in final wound contraction. Pairwise Mann–Whitney U tests were used, with Holm-adjusted p-values to control for multiple comparisons. These comparisons are especially important because the Day 14 values were clustered near complete contraction, making it necessary to determine whether the significant omnibus result was driven by specific group contrasts. As shown in Table 18, only the comparison between the negative control group and the 10% extract group remained statistically significant after adjustment, $U = 0.000$, adjusted $p = 0.017$, Cliff's $\delta = -1.000$. The negative mean difference indicates that the 10% extract group had higher final wound contraction than the negative control group. Because the absolute mean difference was 2.154 percentage points at a near-ceiling endpoint, this finding should be interpreted as evidence of greater final-stage visible contraction relative to the negative control, not as broad evidence of large biological wound-healing superiority.

Table 18 Post Hoc Pairwise Comparisons of Day 14 Wound Contraction.

Day	Comparison	Mean difference	U	Adjusted p	Cliff's δ	Interpretation
Day 14	Negative Control Ointment – Positive Control Ointment	-0.199	16.000	0.810	-0.111	Not significant
Day 14	Negative Control Ointment – 5% Concentration Ointment	-1.904	3.000	0.091	-0.833	Not significant
Day 14	Negative Control Ointment – 10% Concentration Ointment	-2.154	0.000	0.017	-1.000	Significant
Day 14	Positive Control Ointment – 5% Concentration Ointment	-1.705	9.000	0.439	-0.500	Not significant
Day 14	Positive Control Ointment – 10% Concentration Ointment	-1.955	6.000	0.114	-0.667	Not significant
Day 14	5% Concentration Ointment – 10% Concentration Ointment	-0.250	12.000	0.439	-0.333	Not significant

- *Note.* Pairwise comparisons were conducted for Day 14 because the omnibus Kruskal–Wallis test was statistically significant. U = Mann–Whitney test statistic. Adjusted p-values use Holm adjustment. Mean differences are expressed in percentage points and were computed as the first group minus the second group in each comparison. Negative mean differences indicate higher final wound contraction in the second group listed.

Because several Day 14 values were tied at or near 100%, U and Cliff's δ should be interpreted as rank-based final-stage separation rather than as evidence of a large practical wound-healing difference.

The comparison between the negative control group and the 5% extract group did not reach statistical significance after adjustment, adjusted $p = 0.091$, although the direction of the mean difference favored the 5% extract group. Likewise, the positive control group did not differ significantly from either the 5% extract group, adjusted $p = 0.439$, or the 10% extract group, adjusted $p = 0.114$. The direct comparison between the 5% and 10% extract groups was also not statistically significant, adjusted $p = 0.439$.

These post hoc results indicate that the clearest Day 14 group difference was between the negative control and the 10% extract ointment. However, the extract formulations were not statistically superior to the positive control, and the two extract concentrations did not differ significantly from each other. Thus, the Day 14 post hoc results support a cautious conclusion of greater final contraction for the 10% extract relative to the negative control only.

➤ Discussions

The study investigated the potential wound-healing effect of the ethanolic leaf extract of *Sandoricum koetjape* (Meliaceae) using an excision wound model in Swiss Albino mice. Extraction of the dried leaf samples through 90% ethanol maceration produced a mean yield of 14.57% from three 20 g samples, with individual yields of 15.0%, 13.2%, and 15.5%, indicating consistent recovery of extractable constituents from the plant material. Crude extract evaluation revealed a dark greenish-brown color of it, with a distinct herbal odor and a viscous, semi-solid consistency, as shown in Table 9. These characteristics are considered acceptable for subsequent formulation into topical preparations, as these properties support ease of incorporation into a stable topical ointment.

Phytochemical confirmatory tests demonstrated the presence of flavonoids, tannins, saponins, and terpenoids in the extract. These secondary metabolites are strongly associated with wound repair mechanisms. Flavonoids are known for their antioxidant and anti-inflammatory effects, helping reduce oxidative stress at the wound site. Tannins contribute to wound contraction and protective barrier formation, while saponins promote angiogenesis and collagen deposition. Terpenoids are also linked to antimicrobial and anti-inflammatory actions that support tissue regeneration. The detection of these compounds provides a scientific basis for the traditional use of *S. koetjape* in treating cuts and skin injuries.

Safety of the extract for topical use was assessed through the OECD 402 acute dermal toxicity procedure. The study reported no visible lesions or abnormal organ findings during necropsy, and recorded organ measurements remained within expected ranges across treated mice. These observations suggest that the ethanolic leaf extract did not produce evident toxic effects when applied dermally under the study conditions. Establishing this safety profile was essential before proceeding to wound-healing evaluation, as it supports the suitability of the extract for dermal application.

The wound-healing evaluation demonstrated progressive wound closure in all treatment groups throughout the 14-day observation period. Reduction in wound area and the corresponding increase in wound contraction indicated that tissue repair occurred continuously from Day 0 to Day 14. However, the extract-treated groups, particularly the 10% ointment formulation, exhibited more favorable wound-healing outcomes during the later stages of healing. By Day 14, the 10% extract ointment achieved complete mean wound contraction, while the 5% extract ointment also produced near-complete wound closure. These findings suggest that the ethanolic leaf extract of *S. koetjape* possesses promising wound-healing activity when formulated as a topical ointment preparation.

The enhanced wound contraction observed in the extract-treated groups may be attributed to the collective wound-healing properties of the phytochemical constituents present in the plant extract. Flavonoids may have contributed to the accelerated healing through their antioxidant activity, which helps neutralize free radicals generated during inflammation and tissue injury. Reduction of oxidative stress is considered important during wound healing because excessive reactive oxygen species may delay fibroblast proliferation and collagen formation (Zhang et al. 2022). Tannins may also have promoted faster wound contraction through their astringent properties, which help precipitate proteins and form a protective barrier over damaged tissue as reported by Hapid et al. (2024). Furthermore, saponins are reported to stimulate collagen synthesis and angiogenesis, both of which are essential for granulation tissue formation and tissue remodeling. The presence of terpenoids may likewise have supported healing by minimizing microbial contamination and reducing inflammatory responses at the wound site (Morgan Babu et al. 2021).

The findings further suggest that the 10% extract formulation may have provided a greater concentration of bioactive constituents necessary for tissue repair compared with the 5% formulation. Although statistical analysis did not establish a significant dose-dependent relationship between the two extract concentrations, the consistent trend toward higher mean wound contraction in the 10% group indicates a possible concentration-related effect. Increased availability of active phytochemicals in the higher-concentration ointment may have enhanced fibroblast activity, collagen deposition, and epithelial regeneration, thereby contributing to faster visible wound closure.

Despite the promising wound-healing activity observed in the extract-treated groups, statistical analysis showed that significant differences among treatment groups were limited. No statistically significant differences were observed during the early and middle stages of healing on Day 3 and Day 7. This may be attributed to the natural capacity of mice to undergo rapid wound contraction regardless of treatment, which can reduce observable differences between groups during the initial phases of healing. In addition, the relatively small sample size used in the study may have limited the statistical power necessary to detect subtle treatment effects.

A statistically significant difference among groups emerged only on Day 14, wherein the 10% extract ointment demonstrated significantly greater wound contraction compared with the negative control ointment. However, the extract-treated groups were not significantly different from the positive control ointment. This finding suggests that the wound-healing activity of the extract may

be comparable to the standard treatment used in the study. Nevertheless, the absence of statistical superiority over the positive control indicates that further optimization and investigation are still necessary before the extract can be considered equally or more effective than commercially available wound-healing preparations.

The interpretation of the results should also consider the limitations of the study. The wound-healing evaluation focused primarily on wound area reduction and wound contraction as indicators of visible healing. Other important parameters associated with tissue repair, such as histopathological examination, collagen quantification, epithelialization period, tensile strength, and antimicrobial activity, were not assessed. Therefore, while the findings demonstrate promising effects on visible wound closure, they do not fully establish the extent of tissue regeneration and biological healing occurring beneath the wound surface.

Overall, the results of the study support the potential of the ethanolic leaf extract of *Sandoricum koetjape* as a topical wound-healing agent. The observed wound contraction activity, combined with the absence of dermal toxicity and the presence of bioactive phytochemicals associated with tissue repair, suggests that the plant may serve as a promising source of natural compounds for wound management. Further studies involving larger sample sizes, broader concentration ranges, histological analyses, and formulation optimization are recommended to further validate its therapeutic potential and mechanism of action.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

This chapter outlines the summary of findings, presents the conclusions derived from the result of the study, and the recommendations formulated based on the obtained insights.

➤ Summary of Findings

The following summarizes the key findings of the study on the wound-healing efficacy of the ethanolic leaf extract of *Sandoricum koetjape*:

- The ethanolic leaf extract of *S. koetjape* was obtained as a viscous, semi-solid substance with a dark greenish-brown color and a distinct herbal odor, indicating its suitability for formulation into a topical ointment.
- The ethanolic extraction of *S. koetjape* leaves through 90% ethanol maceration produced percentage yields of 15.0%, 13.2%, and 15.5% from the triplicate samples, resulting in a mean percentage yield of 14.57%, indicating consistent extraction efficiency using the selected extraction method.
- Phytochemical analysis confirmed the presence of flavonoids, tannins, saponins, and terpenoids, which are associated with antioxidant, antiinflammatory, antimicrobial, and wound-healing activities.
- The experiment involved the preparation of ointments containing 5% and 10% concentrations of the extract, along with a base ointment as the negative control and a 10% povidone-iodine ointment as the positive control.
- The acute dermal toxicity test showed no observable signs of irritation, lesions, or organ abnormalities, suggesting that the extract was well tolerated following topical application under the conditions of the study.
- Wound-healing evaluation using the excision wound model demonstrated progressive wound closure in all treatment groups throughout the 14-day observation period, as evidenced by decreasing wound area and increasing wound contraction percentages.
- The 10% extract ointment exhibited the greatest wound-healing activity among the extract-treated groups, achieving complete mean wound contraction (100%) by Day 14, while the 5% extract ointment achieved near-complete wound contraction (99.75%).
- Statistical analysis revealed that wound contraction significantly increased over time across all groups, indicating continuous healing progression throughout the observation period. However, no statistically significant overall treatmentgroup effect or treatment-by-day interaction was observed.
- Day-specific analysis showed no statistically significant differences among treatment groups on Day 3 and Day 7. However, a statistically significant difference was observed on Day 14.
- Post hoc analysis revealed that only the 10% extract ointment demonstrated significantly greater wound contraction compared with the negative control ointment on Day 14, suggesting promising final-stage wound-healing activity of the higher-concentration extract formulation.
- No statistically significant difference was observed between the 5% and 10% extract ointments, indicating that although the 10% formulation showed slightly better wound contraction, a statistically confirmed dose-dependent effect was not established in the study.
- The findings suggest that the ethanolic leaf extract of *S. koetjape*, particularly at 10% concentration, possesses promising wound-healing potential and may serve as a candidate for further development as a topical herbal preparation.

➤ Conclusion

Based on the results of the study, it can be concluded that the ethanolic leaf extract of *Sandoricum koetjape* possesses potential wound-healing activity when formulated as a topical ointment. The presence of key phytochemical constituents may contribute to its biological activity, particularly in promoting wound contraction and supporting tissue repair. Furthermore, the acute dermal toxicity findings suggest that the extract was well tolerated following topical application under the conditions of the study.

The excision wound-healing evaluation demonstrated progressive wound closure in all treatment groups throughout the 14-day observation period. Among the extract-treated groups, the 10% ointment formulation exhibited the highest mean wound contraction and achieved complete visible wound closure by Day 14, indicating promising final-stage wound-contraction activity. The 5% extract ointment likewise demonstrated near-complete wound contraction, suggesting beneficial effects on observable wound closure when formulated as a topical preparation.

Statistical analysis further showed that wound contraction significantly increased over time, confirming continuous healing progression during the study period. Although the extract-treated groups were not statistically superior to the positive control ointment, the 10% extract formulation demonstrated significantly greater wound contraction compared with the negative control group during the final stage of healing. However, no statistically significant dose-dependent relationship was established between the 5% and 10% extract formulations.

In relation to the study hypothesis, the null hypothesis was rejected only for the Day 14 comparison between the 10% extract group and the negative control group. The null hypothesis was not rejected for comparisons between the extract-treated groups and the positive control group, nor for the direct comparison between the 5% and 10% extract groups. Accordingly, the study supports

a cautious conclusion that the 10% *S. koetjape* extract ointment demonstrated promising final-stage visible wound contraction activity relative to the negative control, but it does not provide definitive proof of complete wound-healing efficacy, superiority over the standard treatment, or a statistically confirmed dose-response relationship.

Overall, the findings suggest that the ethanolic leaf extract of *S. koetjape*, particularly at 10% concentration, may have potential for topical wound-care applications. Nevertheless, further studies involving larger sample sizes, histopathological analysis, formulation optimization, and additional biological assessments are recommended to further validate its wound-healing potential and therapeutic applicability.

➤ *Recommendations*

Based on the findings and limitations of the study, the following recommendations are proposed:

- **Clinical Formulation Development** – Future pharmaceutical formulations of *S. koetjape* should prioritize optimized concentrations for topical wound-healing products to balance efficacy, cost-effectiveness, and safety.
- **Concentration Optimization** – Researchers should explore a wider range of extract concentrations beyond 5% and 10% to establish a more detailed dose-response relationship and identify the optimal therapeutic strength.
- **Inclusion of Female Subjects** – Future studies should include female mice to evaluate potential gender-related variations in wound-healing responses, ensuring broader applicability of results.
- **Diverse Formulation Types** – Investigate alternative delivery systems such as gels, sprays, or hydrogel dressings to provide more versatile options for different types of skin injuries.
- **Testing on Varied Wound Conditions** – Future studies should assess the performance of *S. koetjape*-based formulations specifically on different wound models, as varied environments can significantly affect absorption and healing recovery.
- **Isolation and Characterization of Active Compounds** – Further research should focus on isolating specific active constituents from *S. koetjape* responsible for the wound-healing effects to better understand their individual contributions.
- **Long-Term Healing and Scar Assessment** – Studies should extend beyond the 14-day observation period to evaluate long-term outcomes such as scar formation and the restoration of skin integrity.
- **Expanded Biological Evaluation** – Further investigations should include histopathological examinations of healed tissues to assess collagen deposition, angiogenesis, and the quality of tissue regeneration at the microscopic level.

REFERENCES

- [1]. Agapin JS. 2020. Medicinal plants used by traditional healers in Pagadian City, Zamboanga del Sur, Philippines. *Philippine Journal of Science* [Internet]. [cited 2025 Aug 31];149(3):643–654. Available from: https://philjournalsci.dost.gov.ph/images/pdf/pjs_pdf/vol149no3/medicinal_plants_pagadian.
- [2]. Akinmoladun FO, Komolafe TR, Komolafe OA, Farombi EO. 2021. Phytochemical screening and wound healing activity of *Azadirachta indica* leaf extract in Wistar rats. *J Herbmmed Pharmacol* [Internet]. [cited 2025 Aug 31]; 10(2):166-172. Available from: doi:10.34172/jhp.2021.18.
- [3]. Alvarez-Peral J, Arrieta-Baez D, et al. 2023. Natural products and skin regeneration: advances in wound healing. *Int J Mol Sci* [Internet]. [cited 2025 Aug 27]; 24(2):1154. Available from: doi: 10.3390/molecules28020598.
- [4]. Alvian D, Susilo RJ, Pratiwi D. 2021. Ethnomedicinal, phytochemicals, and pharmacological aspects of sentul (*Sandoricum koetjape*). *Biomedich J Sci Tech Res* [Internet]. [cited 2025 Sep 26]; Available from: <https://sciencebiology.org/index.php/biomedich/article/view/177>
- [5]. Armaghan M, Khushbukhat K, Irfan M, Hafeez A, Zafar S, Javed Z, Javad S, Butnariu M, Sarac I, Bagiu I, et al. 2024. Koetjapic acid: unveiling its potential as a saviour in the realm of biological and medicinal properties, with a focus on anticancer mechanism of action. *Eur J Med Res* [Internet]. [cited 2025 Sep 8];29(1). Available from: doi:<https://doi.org/10.1186/s40001-024-01699-6>.
- [6]. Alrouji M, Kuriri FA, Alqasm MH, AlSudais H, Alissa M, Alsuwat MA, Asad M, Joseph B, Almuhan Y. 2023. A simple in-vivo method for evaluation of antibiofilm and wound healing activity using excision wound model in diabetic Swiss Albino mice. *Microorganisms* [Internet]. [cited 2025 Oct 12];11(3) 692. Available from: <https://doi.org/10.3390/microorganisms11030692>.
- [7]. Awolola GV, Jinadu TH, Yakubu MT, Oyewopo AO, Habibur RM. 2023. In-vivo therapeutic evaluation of the wound healing potential of ethanolic extract of *Trichilia heudelotii* leaves in Wistar rats [Internet]. [cited 2025 Sep 6]. Available from: <https://www.researchgate.net/publication/376565698>.
- [8]. Bailly C. 2022. Exploring the use of herbal drugs and advanced supporting techniques for wound healing. *Bull Natl Res Cent* [Internet]. [cited 2025 Aug 27]; 46:16. Available from: <https://doi.org/10.1186/s42269-022-00705-3>.
- [9]. Bailly C. 2022. The health benefits of santol fruits and bioactive products isolated from *Sandoricum koetjape* Merr.: a scoping review. *J Food Biochem* [Internet]. [cited 2025 Aug 28];46(7):e14152. Available from: doi: 10.1111/jfbc.14152.
- [10]. Baron JM, Glatz M, Proksch E. 2020. Optimal support of wound healing: new insights. *Dermatology* [Internet]. [cited 2025 Sep 7];236(6):593–600. Available from: <https://doi.org/10.1159/000505291>.
- [11]. Bowers S, Franco E. 2020. Chronic wounds: evaluation and management. *Am FamPhysician* [Internet]. [cited 2025 Sep 7];101(3):159–166. Available from: <https://pubmed.ncbi.nlm.nih.gov/32003952/>
- [12]. Cardoso FCI, Bortolozo BB, Azevedo FF, Zanchetta FC, Morari J, Silva GT de S, Rosa PCP, Araujo EP, Lima MHM. 2025. Effect of *Plantago major* on wound healing in hyperglycemic mice. *Journal of Biosciences and Medicines* [Internet]. [cited 2025 Aug 29]; 13(04):320–333. Available from: doi:<https://doi.org/10.4236/jbm.2025.134026>.
- [13]. Cedillo-Cortezano M, Martinez-Cuevas LR, Márquez López JA, Barrera López IL, Escutia-Perez S, Petricevich VL. 2024. Use of medicinal plants in the process of wound healing: a literature review. *Pharmaceuticals* [Internet]. [cited 2025 Aug 27]; 17(3):303. Available from: <https://doi.org/10.3390/ph17030303>.
- [14]. Chelms-Burlacu A, Profire L, Pieptu D. 2023. An up-to-date on povidone-iodine with focus on wound healing management: current available data and new approaches. *Farmacia Journal* [Internet]. [cited 2025 Sept 8]. Available from: <https://farmaciajournal.com/issue-articles/an-up-to-date-on-povidone-iodine-with-focus-on-wound-managemnt-current-available-data-and-new-approaches/>.
- [15]. Choudhary V, Choudhary M, Bollag WB. 2024. Exploring skin wound healing models and the impact of natural lipids on the healing process. *Int J Mol Sci* [Internet]. [cited 2025 Sep 9];25(7):3790. Available from: doi: 10.3390/ijms25073790.
- [16]. Criollo-Mendoza MS, Contreras-Angulo LA, Leyva-López N, Gutiérrez-Grijalva EP, Jiménez-Ortega LA, Heredia JB. 2024. Wound healing properties of natural products: mechanisms of action. *Molecules* [Internet]. [cited 2025 Aug 27]; 2023;28(2):598. Available from: <https://doi.org/10.3390/molecules28020598>.
- [17]. Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S. 2023. Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in ethiopia. *J Exp Pharmacol* [Internet]. [cited 2025 Sept 25];15:51-62. Available from: doi: 10.2147/JEP.S379805.
- [18]. Durani P, Leaper D. 2024. Povidone–iodine: use in hand disinfection, skin preparation, and antiseptic irrigation. *Int Wound J* [Internet]. [cited 2025 Sep 8]. Available from: <https://onlinelibrary.wiley.com/doi/10.1111/j.1742-481X.2007.00405>.
- [19]. Fajri R, et al. 2021. Angiogenic modulatory activity of *Sandoricum koetjape* ethanolic leaf extract observed through duck embryo CAM assay. *Biomed Pharmacol J* [Internet]. [cited 2025 Sep 26]; Available from: <https://www.biomedscidirect.com>
- [20]. Fernández-Guarino M, Hernández-Bule ML, Bacci S. 2023. Cellular and molecular processes in wound healing. *Biomedicines* [Internet]. [cited 2025 Aug 28];11(9):2526. Available from: doi:10.3390/biomedicines11092526.
- [21]. Forestry. 2024 Oct 31. Philippine Santol Tree [Internet]. [cited 2025 Aug 29]. Available from: <https://forestry.com/trees/philippine-santol-tree>.

- [22]. Gao M, Guo H, Dong X, Wang Z, Yang Z, Shang Q, Wang Q. 2024. Regulation of inflammation during wound healing: the function of mesenchymal stem cells and strategies for therapeutic enhancement. *Front Pharmacol*. [cited 2025 Sep 7];15:1345779. Available from: <https://doi.org/10.3389/fphar.2024.1345779>
- [23]. Gmur MK, Karpiński TM. 2020. Povidone-iodine in wound healing and prevention of wound infections. *Eur J Biol Res*. Available from: <http://www.journals.tmkarpinski.com/index.php/ejbr/article/view/325/148>
- [24]. Grubbs H, Manna B. 2023. Wound physiology. [Internet] [cited 2025 Sep 7]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK518964/>
- [25]. Girgis C, Suludere MA, Crisologo PA, Lavery LA. 2023 Medical mythology, misconceptions, and misinformation: does iodine impede wound healing? *Wounds* [Internet]. [cited 2025 Sep 8];35(9):E297-E305. Available from: doi: 10.25270/wnds/23047. PMID: 37769289.
- [26]. Hamzah FN, et al. 2024. Investigating Sandoricum koetjape leaf extracts for tyrosinase inhibitors: in vitro and in silico study. *E3S Web Conf. (ICAS2024)* [Internet]. [cited 2025 Aug 28]; 577,02008. Available from: <https://doi.org/10.1051/e3sconf/202457702008>.
- [27]. International Plant Names Index (IPNI). 2024. *Sandoricum koetjape* (Burm.f.) Merr. Published in Philipp. J. Sci., C 7:237 (1912). [Internet] [cited 2025 Aug 29]. Available from: <https://www.ipni.org>
- [28]. Kamrani P, Hedrick J, Marks JG, Zaenglein AL. Petroleum jelly: A comprehensive review of its history, uses, and safety. *J Am Acad Dermatol* [Internet]. [cited 2025 Aug 29];90(4):807-813. Available from: <https://pubmed.ncbi.nlm.nih.gov/37315800/>
- [29]. Krakowska-Sieprawska A, Kosmalka A, Kurek MA. 2022. Modern methods of pre-treatment of plant material for the extraction of bioactive compounds. *Molecules*. 27(3):8840492. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC8840492/>
- [30]. Liu E, Gao H, Zhao Y, Pang Y, Yao Y, Yang Z, Zhang X, Wang Y, Yang S, Ma X, et al. 2022. The potential application of natural products in cutaneous wound healing: a review of preclinical evidence. *Front Pharmacol* [Internet]. [cited 2025 Aug 27]; 13:900439. Available from: <https://doi.org/10.3389/fphar>.
- [31]. Mathew-Steiner SS, Roy S, Sen CK. 2021. Collagen in wound healing. *Bioengineering (Basel)* [Internet]. [cited 2025 Sep 7];8(5):63. Available from: <https://doi.org/10.3390/bioengineering8050063>
- [32]. Monstrey SJ, Govaers K, Lejoste P, Lepelletier D, Ribeiro PO. 2023. Evaluation of the role of povidone-iodine in the prevention of surgical site infections. *Surgery Open Science* [Internet]. [cited 2025 Aug 29];13:9–17. Available from: doi: <https://doi.org/10.1016/j.sopen.2023.03.005>.
- [33]. Mogan Babu N, Harikrishnan N, 2020. Wound Healing Potential of Selected Drug Molecules Obtained From Terpenoids [December 2020 *International Journal of Research in Pharmaceutical Sciences*] 11(4):7345-7352 DOI:10.26452/ijrps.v11i4.3915 Available from: https://www.researchgate.net/publication/348270023_Wound_Healing_Potential_of_Selected_Drug_molecules_Obtained_From_Terpenoids
- [34]. MSD Manuals. 2025. Wounds. MSD Manual Consumer Version [Internet]. [cited 2025 Sep 8]. Available from: <https://www.msdmanuals.com/home/injuries-and-poisoning/first-aid/wounds>
- [35]. National Parks Board (NParks). 2021. *Sandoricum koetjape* (Santol) [Internet]. [cited 2025 Aug 29]. Available from: <https://www.nparks.gov.sg/florafaunaweb/flora/3/1/3109>
- [36]. Nagle M, et al. 2023. Wound assessment and management. [Internet] [cited 2025 Sep 7]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK482198/>
- [37]. Nguyen HM, Le TTN, Nguyen AT, Le HNT, Pham TT. 2023. Biomedical materials for wound dressing: recent advances and applications. *RSC Adv* [Internet]. [cited 2025 Sep 7];13:5509–28. Available from: <https://doi.org/10.1039/D2RA07673J>
- [38]. Olatunji, T.L., Odebunmi, C.A. & Adetunji, A.E. Biological activities of limonoids in the Genus *Khaya* (Meliaceae): a review. *Futur J Pharm Sci* 7, 74 (2021). <https://doi.org/10.1186/s43094-021-00197-4> from: <https://fjps.springeropen.com/articles/10.1186/s43094-021-00197-4>
- [39]. Park J, De P. 2025. Wound progression and complications. *Int Wound J* [Internet]. [cited 2025 Sep 7]. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1111/iwj.14339>
- [40]. Philippine Traditional Knowledge Digital Library (TKDL). 2025. Santol (*Sandoricum koetjape*). [Internet]. [cited 2025 Aug 31]. Available from: <https://tkdlph.com/index.php/ct-menu-item-3/ct-menu-item-7/all/3396-santol>
- [41]. Rashid S, Majeed LR, Nisar B, Nisar H, Bhat AA, Ganai BA. 2021. Chapter 1 - phytomedicines: diversity, extraction, and conservation strategies. *Phytomedicine* [Internet]. [cited 2025 Sept 25]; 1-33. Available from: <https://doi.org/10.1016/B978-0-12-824109-7.00009-1>.
- [42]. Rahman A, Yusuf S, Putri A. 2021. Ethnobotanical survey of medicinal plants used for skin and soft tissue injuries in West Java, Indonesia. *Journal of Ethnopharmacology* [Internet]. [cited 2025 Aug 31];276:114178. Available from: <https://doi.org/10.1016/j.jep.2021.114178>
- [43]. Saadah S, Tulandi SM, Rohman RA. 2022. Phytochemical and gas chromatography-mass spectrometry profiling of two plant parts of *Sandoricum koetjape*. *Biodiversitas* [Internet]. [cited 2025 Sept 25]; 23(12):6199-6207. Available from: doi:10.13057/biodiv/d231214.
- [44]. Santos MG, et al. 2020. Antibacterial activity of *Sandoricum koetjape* leaf ethanolic extract. *DLSMHSI J Res* [Internet]. [cited 2025 Sep 26]; Available from: https://greenprints.dlshsi.edu.ph/grade_12/104

- [45]. Simon PE, Al Moutran H, Romo T III, et al. 2024. Skin wound healing: overview, hemostasis, inflammatory, proliferative, and remodeling phases. Medscape [Internet]. [cited 2025 Sep 7]. Available from: <https://emedicine.medscape.com/article/884594>
- [46]. Shewaye DG, Kahaliw W, Belete TM, Ahmed N. 2023. Evaluation of wound healing and anti-inflammatory activities of 80% methanol crude extract and solvent fractions of *Trichilia dregeana* (Meliaceae) leaves in mice. Evid Based Complement Alternat Med [Internet]. [cited 2025 Aug 27]; 9980866. Available from: doi: 10.1155/2023/9980866.
- [47]. Uberoi A, McCready-Vangi A, Grice EA. 2024. The wound microbiota: microbial mechanisms of impaired wound healing and infection. Nat Rev Microbiol [Internet]. [cited 2025 Sep 7];22(8):507–521. Available from: <https://doi.org/10.1038/s41579-024-01035-z>
- [48]. West Coast Wound. 2025. Traumatic wound treatment and care. West Coast Wound [Internet]. [cited 2025 Sep 8]. Available from: <https://westcoastwound.com/traumatic-wound-treatment-and-care>
- [49]. Wijaya MD. 2022. Ethnomedicinal, phytochemicals, and pharmacological aspects of Sentul (*Sandoricum koetjape*). Biol Med Nat Prod Chem [Internet]. [cited 2025 Aug 28]; 11(1):177. Available from: <https://sciencebiology.org/index.php/BIOMEDICH/article/view/177>.
- [50]. World Flora Online (WFO). 2024. *Sandoricum koetjape* (Burm.f.) Merr. World Flora Online Consortium. [Internet] [cited 2025 Aug 29]. Available from: <http://www.worldfloraonline.org>
- [51]. World Flora Online (WFO). 2024. *Sandoricum koetjape* (Burm.f.) Merr. World Flora Online Consortium. [Internet] [cited 2025 Aug 29]. Available from: <http://www.worldfloraonline.org>.
- [52]. Wuhan Diao Pharmaceutical Co. Ltd. 2023. CN102885852B-Povidone iodine ointment and preparation method thereof. Google Patents. [Internet] [cited 2026 March 30]. Available from: <https://patents.google.com/patent/CN102885852B/en>.
- [53]. Wutsqa YU, Suratman S, Sari LA. 2021. Detection of terpenoids and steroids in *Lindsaea obtusa* with thin layer chromatography. Asian J Nat Prod Biochem [Internet]. [cited 2025 Sept 25]; 19(2):66-69. Available from: doi: 10.13057/biofar/f190204.
- [54]. Yang Y, Huang J, Zeng A, Long X, Yu N, Wang X. 2024. The role of the skin microbiome in wound healing. Burns Trauma [Internet]. [cited 2025 Sep 7];12:tkad059. Available from: <https://doi.org/10.1093/burnst/tkad059>
- [55]. Yassine KA, Houari H, Mokhtar B, Karim A, Hadjer S, Bediaf I. 2020. A topical ointment formulation containing leaves' powder of *Lawsonia inermis* accelerate excision wound healing in Wistar rats. J Ethnopharmacol [Internet]. [cited 2025 Sep 26]; Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7429373>
- [56]. Zhang X, et al. 2022. Phytochemical-based nanoformulations for wound healing: current applications and innovations. Appl Sci [Internet]. [cited 2025 Aug 28]; 12(24):12654. Available from: doi: 10.3390/gels7040209.
- [57]. (Rahmawati R, Nurjanah M, Yuliani R, et al. Antioxidant and antibacterial activities of *Sandoricum koetjape* leaf extract. J Appl Pharm Sci. 2022;12(7):142-148.)

APPENDIX A SCHEDULE OF ACTIVITIES

- *Project Dates: January 2026 to May 2026*

	RESEARCH OBJECTIVES	2026				
		JAN	FEB	MAR	APR	MAY
1	Plant Collection					
2	Plant Authentication					
3	Requesting and Purchasing of Chemical and Reagents to be Used					
4	Purchasing of Mice					
5	Blending and Maceration of Plant Extract					
6	Phytochemical Testing					
7	Formulation of OECD					
8	Administration of OECD					
9	Formulation of Positive and Negative Control					
10	Formulation of <i>Sandoricum koetjape</i> (5% and 10%)					
11	Administration of Ointment Preparations					
12	Writing of Chapter IV: Results and Discussion					
14	Writing of Chapter V: Conclusion and Recommendations					
15	Final Defense hearing					
16	Revision of Proposal Manuscript According to Thesis Panel Suggestions, Recommendations, and Annotations					
17	Submission of Approved and Signed Final Manuscript					

APPENDIX B

CERTIFICATE OF ANALYSIS (ETHANOL)



Product Specification

Product Name: ETHANOL Undenatured 100% LR

Alternate Name(s) Ethyl alcohol.

Description

Clear, Colourless liquid.

Properties

Chemical Formula: C_2H_5OH

Molecular Weight: 46.07

Product Code: EL043

CAS No.: 64-17-5

General Information:

Hygroscopic, miscible with water, methyl alcohol, ether, chloroform and acetone.
Flammable; dangerous fire risk.

Hazard and Safety Data

UN Group: II
Class: 3
UN Number: 1170
Hazchem code: +2YE
CS MSDS Code: 1CH89
Poison schedule: Not Scheduled
Emergency
Procedure Guide No.: 14

Quality Specification

Assay: 99.5% min.

Specific Properties and Impurities [Typical levels]:

Water	≤ 0.5%
Colour (APHA)	≤ 10
Residue after evaporation	≤ 0.0025%
Solubility in water	Passes test
Acidity (as acetic acid)	≤ 0.0030%
Substances darkened by sulfuric acid	Passes test
Substances reducing permanganate	Passes test
Volatile Impurities	
- Methanol	≤ 0.0150%
- Acetaldehyde and acetal	≤ 0.0015%
- Benzene	≤ 0.0002%
- Sum of other impurities	≤ 0.0300%
Aluminium (Al)	≤ 0.00001%
Barium (Ba)	≤ 0.00001%
Cadmium (Cd)	≤ 0.00001%
Calcium (Ca)	≤ 0.00002%
Chromium (Cr)	≤ 0.00001%
Cobalt (Co)	≤ 0.00001%
Copper (Cu)	≤ 0.00001%
Iron (Fe)	≤ 0.00002%
Lead (Pb)	≤ 0.00001%
Magnesium (Mg)	≤ 0.00002%
Manganese (Mn)	≤ 0.00001%
Nickel (Ni)	≤ 0.00001%
Potassium (K)	≤ 0.0001%
Sodium (Na)	≤ 0.0005%

Chem-Supply Pty Ltd - An ISO 9001 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

Chem-Supply does not warrant that this product is suitable for any use or purpose. The user must ascertain the suitability of the product for any intended purpose. Preliminary testing of the product before use or application is recommended. Any reliance or purported reliance upon Chem-Supply Pty Ltd with respect to any skill or judgement or advice in relation to the suitability of this product for any purpose is disclaimed. Except to the extent prohibited at law, any condition implied by any statute as to the merchantable quality of this product or fitness for any purpose is hereby excluded. This product is not sold by description. Where the provisions of Part V, Division 2 of the Trade Practices Act apply, the liability of Chem-Supply Pty Ltd is limited to the replacement or supply of equivalent goods or payment of the cost of replacing the goods or acquiring equivalent goods.

Rev. No: 5

APPENDIX C
CERTIFICATE OF ANALYSIS (DICHLOROMETHANE)



Dichloromethane, CG

Reagent for general industrial purposes.

CH ₂ Cl ₂	FW. 84.93
Code	05S0006
CAS-No.	75-09-2
Density 1 L =	1.330 Kg.
Boiling Point	40 °C
Melting Point	-95 °C

Description

Assay (by GC.)
Appearance
Color (APHA)
Water (by Coulometry)
Acidity (as Hydrochloric Acid)

Specifications

99.8%	min.
Clear liquid	
10	max.
0.1%	max.
0.0005%	max.

Stabilized with about 50 ppm Amylene.

APPENDIX D

CERTIFICATE OF ANALYSIS (PETROLEUM JELLY)


**LABORATORY REAGENTS
& FINE CHEMICALS**
 ISO 9001-2015 CERTIFIED

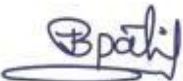
CERTIFICATE OF ANALYSIS

Product Name : PETROLEUM JELLY WHITE Extra Pure Used in cosmetic aid & lubricant.
Release Date : 30-Jan-2026
Mol. Formula : **Mol. Weight** :
Code No. : 05158 **CAS No.** : 8009-03-8
Mfg. Date : Jan-2026 **Exp. Date** : Dec-2030
HAZ. / P.G. : - **UN No.** : -

Sr.	Tests	Specifications	Results
1	Appearance	A snow white jelly	A snow white jelly
2	Melting point	38 - 55°C	49.8°C
3	UV absorbance (0.05% in iso octane at 290 nm)	Max 0.75	0.022%

CONCLUSION -This above product complies as per the specifications of **LOBA CHEMIE PVT. LTD.**


 Monil Tandel
 Chemist


 Jagruti B. Patil
 QC Manager

LOBA CHEMIE PVT. LTD.

Mfg. Add.: LOBA Chemie Pvt. Ltd. Plot No. D-22, MIDC Tarapur, Boisar, Tal. & Dist.-Palghar, Maharashtra-401506
 Tel: 91.02525-663630/39/34
 HO Add.: Loba Chemie Pvt. Ltd., Jehangir Villa, 107, Wodehouse Road., Colaba, Mumbai, Maharashtra – 400005
 Customer Care No: +9122 66636663/Email:info@lobachemie.com www.lobachemie.com

LOBA/QC/FM/01
07/27.10.2025

APPENDIX E

CERTIFICATE OF ANALYSIS (POVIDONE-IODINE)



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	N25112069	数量 Quantity	100G
生产日期 Manufacture Date	2025.03.28	有效时间 Expired Date	2028.03.27
检测日期 Test Date	2025.03.29	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%	3.70%	
AVAILABLE IODINE	9-12%	10.6%	
PH	1.5-5	3.2	
IODIDE ION	≤6.6%	2.9%	
SULFATE ASH	≤0.025%	0.018%	
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):

程勇

审核

(CHECKED):

朱雨昕

质量经理

(QC MANAGER):



APPENDIX F

PLANT AUTHENTICATION

DEPARTMENT OF BIOLOGICAL SCIENCES
COLLEGE OF SCIENCE AND MATHEMATICS

esm.biosci@msuilit.edu.ph

www.msuit.edu.ph



CERTIFICATE OF AUTHENTICATION OF BOTANICAL SPECIMENS

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

Name: **Princess Dona Bagonggong**

Faith Mañabo

Ron Arhiz D. Monteclaro

Maureen Perandos

School/Institution: **Adventist Medical Center College – Iligan City**

Family	Scientific Name	Local/Common Name
Meliaceae	<i>Sandoricum koetjape</i> (Burm.f.) Merr.	Cotton fruit(Eng), santol(MnyLgs)

Collected from: Brgy. Santiago, Iligan City

Date of Collection: 15 January 2026


Muhmin Michael E. Mañting, MSc.

Assistant Professor

Department of Biological Sciences

MSU-Iligan Institute of Technology

APPENDIX G
VETERINARY CERTIFICATE



Veterinary Certificate

Eldani S. Fernando
Veterinarian
Furpet'sake Veterinary Clinic
Miguel sheker, Bagong Silang, Iligan City. 9200
(063) +9171050446
furpetsakeclinic@gmail.com

January 29, 2026

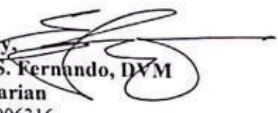
To Whom It May Concern,

And to the best of my knowledge and ability, find the animals at the time of examination to be apparently free from evidence of dangerous communicable animal diseases and external parasites and found to be apparently healthy.

OWNED BY:	Princess Dona C. Baganggong Faith S. Mañabo Arhiz Ron O. Monteclaro Maureen Kathleen L. Perendos
ADDRESS:	Suarez, Iligan City.
CONTACT NUMBER:	09535908588
EMAIL ADDRESS:	bagonggong.princess@amcc.edu.ph

Description:

Name of pet:	Albino Mice
Species:	Mus musculus
Breed:	outbred mice (no specific strain)
Source:	local breeder
Gender:	Male
Color:	White
Weight:	N/A
Birthday:	N/A
Microchip #:	N/A

Sincerely,

Eldani S. Fernando, DVM
Veterinarian
PRC # 0006316

APPENDIX H

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 : <i>AR - 2026 - 0306</i>
	DATE/VENUE : May 2026 - June 2026 AMCC Laboratory
BUSINESS ADDRESS : Brgy. San Miguel, Iligan City	LEAD RESEARCHER/VETERINARIAN/IACUC CHAIR: Bagonggong, Princess Dona C., et.al. - Researcher Loralie G. Oclarit, DVM - Veterinarian Roselle Remulta-Lauron, RPh, MSPH - 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/procedure entitled “Potential Wound Healing Effect of Ethanolic Leaf Extract of <i>Sandoricum koetjape</i> (Meliaceae) in Excision Wound Model in Swiss Albino Mice” on the date and venue stipulated above. The Institution is hereby reminded to observe the provisions of DA-AO no. 40 s.1999. Prepared on May 17, 2026	
Approved By Authority of the Director  JOANNA MARIE F. DAVID, DVM Veterinarian III Animal Health and Welfare Division	
RF AHWD-49 Animal Research Clearance Rev. No. 03 April 19, 2023	

APPENDIX I

CERTIFICATION FOR STATISTICIAN ANALYSIS



CERTIFICATION FOR STATISTICAL ANALYSIS

This is to certify that the study titled *"Potential Wound Healing Effect of Ethanolic Leaf Extract of Sandoricum koetjape (Meliaceae) in Excision Wound Model in Swiss Albino Mice"* conducted by **Princess Dona C. Bagonggong, Faith S. Mañabo, Ron Arhiz D. Monteclaro, and Maureen Kathleen L. Perandos**, Bachelor of Science in Pharmacy students of Adventist Medical Center College–Iligan City, has undergone statistical analysis performed by the undersigned, **Rey R. Cuenca, MSc**, as the statistician.

This certification is issued at the request of the researchers as part of their defense requirements.

Issued this 10th day of May 2026.

Signed,

Rey R. Cuenca, MSc
Statistician



By authority of the Republic of the Philippines, the Board of Regents,
on the recommendation of the University Council, has conferred on

REY R. CUENCA
the **DEGREE** of
Master of Science in Statistics

with all its Rights and Honors as well as the Duties and Obligations pertaining thereto. In testimony whereof are affixed on this 10th day of May, 2026, the Seal of Mindanao State University-Iligan Institute of Technology and the signatures of the President of the University and of the Chancellor and the Registrar of the Institute.



CHARTERED FREE CERTIFICATE
JULIETH P. VALERA, Ph.D.
REGISTRAR

10th day of May, 2026, at Iligan City, Republic of the Philippines.

10th day of May, 2026, at Iligan City, Republic of the Philippines.

10th day of May, 2026, at Iligan City, Republic of the Philippines.

10th day of May, 2026, at Iligan City, Republic of the Philippines.

10th day of May, 2026, at Iligan City, Republic of the Philippines.

10th day of May, 2026, at Iligan City, Republic of the Philippines.

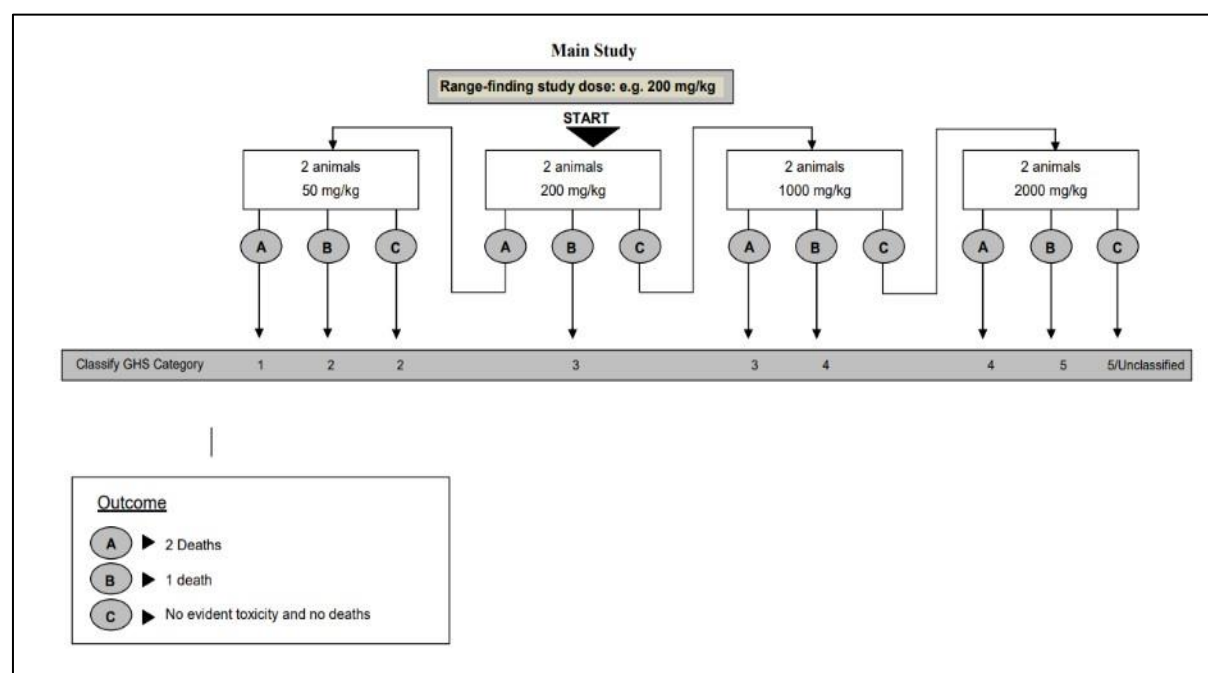
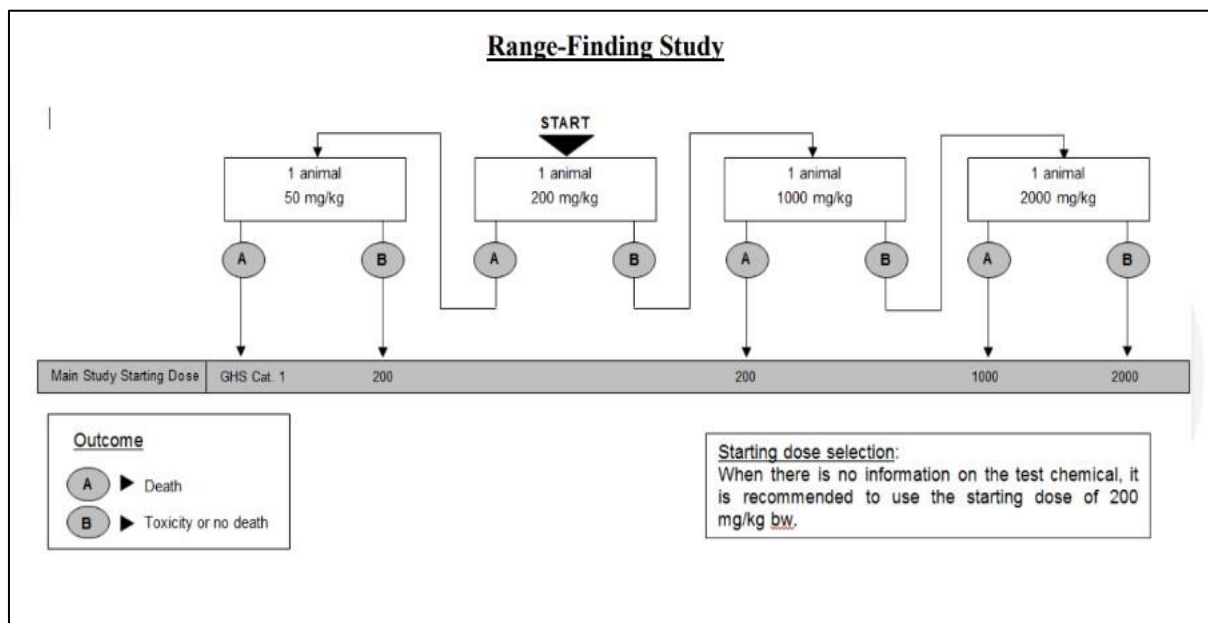
10th day of May, 2026, at Iligan City, Republic of the Philippines.

Note:

- This certification is authenticated through the QR code provided above.
- The official e-copy is exclusively owned and maintained by the statistician at rey.cuenca@g.msuiit.edu.ph.
- Certifications lacking this footer, QR code verification, or confirmed ownership via the drive link of rey.cuenca@g.msuiit.edu.ph are invalid.
- Report suspected forgery immediately to rey.cuenca@g.msuiit.edu.ph.

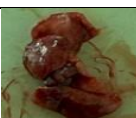
APPENDIX J

OECD 402



➤ *Mice Organs in OECD 402 Acute Dermal Toxicity Test with Crude Extract*

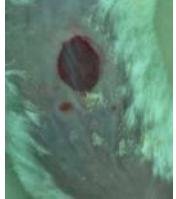
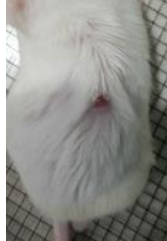
MICE ID	Lungs	Spleen	Kidneys	Liver
M1 (50 mg/kg)				
M1 (200 mg/kg)				

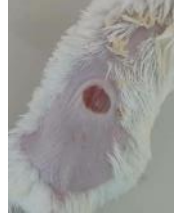
M1 (1000 mg/kg)				
M1 (2000 mg/kg)				
M2 (2000 mg/kg)				
M3 (2000 mg/kg)				

➤ *Mice Organs in OECD 402 Acute Dermal Toxicity Test with S. koetjape leaf extract Ointment*

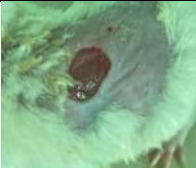
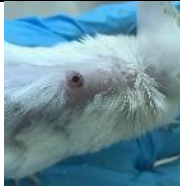
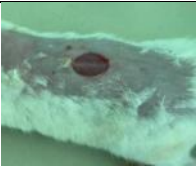
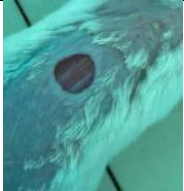
MICE ID	Lungs	Spleen	Kidneys	Liver
M1 (5000 mg/kg)				

➤ *Wound Healing in Mice Treated with Positive Control Ointment*

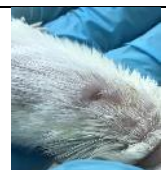
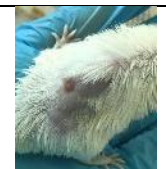
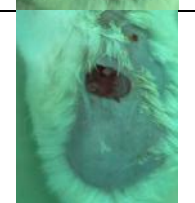
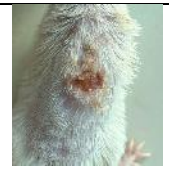
MICE ID	DAY 0	DAY 3	DAY 7	DAY 14
M1				
M2				
M3				
M4				

M5				
M6				

➤ *Wound Healing in Mice Treated with Negative Control Ointment*

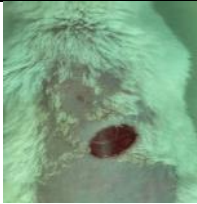
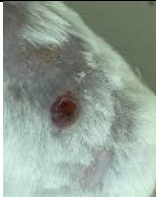
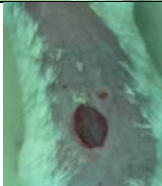
MICE ID	DAY 0	DAY 3	DAY 7	DAY 14
M1				
M2				
M3				
M4				
M5				
M6				

➤ Wound Healing in Mice Treated with 5% Ointment

MICE ID	DAY 0	DAY 3	DAY 7	DAY 14
M1				
M2				
M3				
M4				
M5				
M6				

➤ Wound Healing in Mice Treated with 10% Ointment

MICE ID	DAY 0	DAY 3	DAY 7	DAY 14
M1				
M2				

M3				
M4				
M5				
M6				

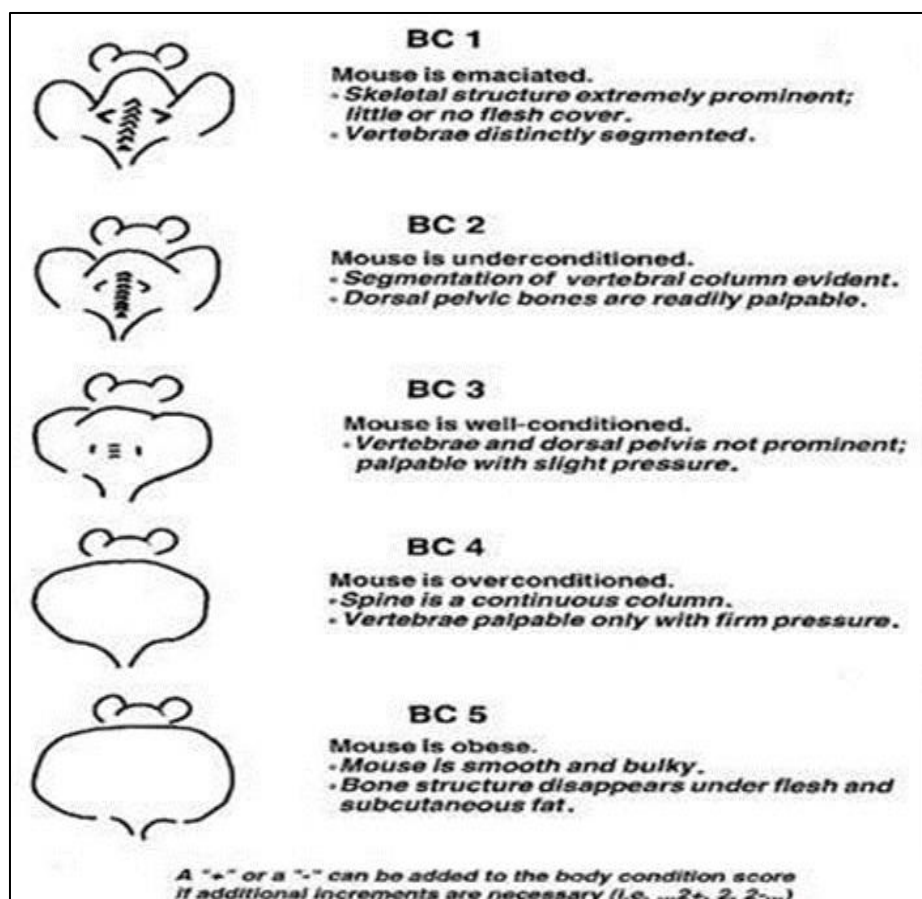
APPENDIX K RAW DATA

➤ Toxicity Observations (OECD 402)

➤ Scoring Criteria for Mice

These parameters served as basis for evaluation during gross necropsy.

Score criteria	0	1	2	3
Weight	Normal, no decrease, mouse is well fleshed and bones are barely felt	Markedly reduced loss of 5% over 24 hours	Weight loss > 15% (or up to 10% in 24 hours)	Weight loss > 10% over 24 hours or > 20% over time
Dehydration	Normal	Skin less elastic	Skin tents	Skin tents, eyes sunken
Movement	Normal	Slight incoordination/ abnormality	Uncoordinated, altered gait, reluctant to move	Staggering, paralysis, limb dragging
Posture	Normal	Slight hunch	Hunched, still moving normally	Severely hunched, impairing movement
Coat/skin condition	Normal	Rough/ lack of grooming	Unkempt, thin, starring coat, flaky skin	Thickened ski, scabby, wounds, fur missing
Breathing	Normal	Rapid, shallow	Rapid, abdominal	Labored, pale, MM or blue
Presence of feces/urine	Normal, feces and urine present, food and water stock decreased in hopper (on cage base and bottle)	Moist feces	Diarrhea or dry feces, abnormal urine (volume/color) no urine or feces present	Uncontrolled diarrhea, blood in feces. Nil urine or incontinent, or no urine or feces produced in 24 hours
Nest quality	Deep nest constructed using available material, nest being utilized	Nest constructed and in use but is thin and lacks shape and depth	Utilizes nesting material to rest on but has not shredded to construct a proper nest	No nest building activity, no attempt has been made to move or utilize nesting material



➤ Toxicity Observation Sheet

Toxicity Observation Sheet Toxicity study of <i>Sandoricum Koetjape</i> extract							
Animal Number: 1	Treatment Group: Extract		Dose received: 50 mg/kg		Date and Time the dose was given: March 05, 2026 – 1:00 pm		
Initial Weight: 30.7 g							
	1st 30 minutes	1 hour	2 H	3H	4H	24 H	Day 14
Weight	30.7 g	30.7 g	30.7 g	30.7 g	30.7 g	30.7 g	35 g
Movement	0	0	0	0	0	0	0
Coat Condition	0	0	0	0	0	0	0
Posture	0	0	0	0	0	0	0
Breathing	0	0	0	0	0	0	0
Feces and Urine	0	0	0	0	0	0	0
Attitude	0	0	0	0	0	0	0
Appearance	0	0	0	0	0	0	0
BCS	0	0	0	0	0	0	0
Specific Clinical Signs							
Tremors	0	0	0	0	0	0	0
Circling	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0
Comatose	0	0	0	0	0	0	0
Salivations	0	0	0	0	0	0	0
<u>Other observations:</u>							

<u>Toxicity Observation Sheet</u> Toxicity study of <i>Sandoricum Koetjape</i> extract							
<u>Animal Number:</u> 2	<u>Treatment Group:</u> Extract		<u>Dose received:</u> 200 mg/kg		<u>Date and Time the dose was given:</u> March 05, 2026 – 1:00 pm		
Initial Weight: 24.4 g							
	1st 30 minute s	1 hour	2 H	3H	4H	24 H	Day 14
Weight	24.4 g	24.4 g	24.4 g	24.4 g	24.4 g	24.4 g	28.5 g
Movement	0	0	0	0	0	0	0
Coat Condition	0	0	0	0	0	0	0
Posture	0	0	0	0	0	0	0
Breathing	0	0	0	0	0	0	0
Feces and Urine	0	0	0	0	0	0	0
Attitude	0	0	0	0	0	0	0
Appearance	0	0	0	0	0	0	0
BCS	0	0	0	0	0	0	0
<u>Specific Clinical Signs</u>							
Tremors	0	0	0	0	0	0	0
Circling	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0
Comatose	0	0	0	0	0	0	0
Salivations	0	0	0	0	0	0	0
<u>Other observations:</u>							

Toxicity Observation Sheet							
Toxicity study of <i>Sandoricum Koetjape</i> extract							
<u>Animal Number:</u> 3	<u>Treatment Group:</u> Extract		<u>Dose received:</u> 1000 mg/kg		<u>Date and Time the dose was given:</u> March 05, 2026 – 1:00 pm		
Initial Weight: 26.2 g							
	1st 30 minute s	1 hour	2 H	3H	4H	24 H	Day 14
Weight	26.2 g	26.2 g	26.2 g	26.2 g	26.2 g	26.2 g	30.2 g
Movement	0	0	0	0	0	0	0
Coat Condition	0	0	0	0	0	0	0
Posture	0	0	0	0	0	0	0
Breathing	0	0	0	0	0	0	0
Feces and Urine	0	0	0	0	0	0	0
Attitude	0	0	0	0	0	0	0
Appearance	0	0	0	0	0	0	0
BCS	0	0	0	0	0	0	0
<u>Specific Clinical Signs</u>							
Tremors	0	0	0	0	0	0	0
Circling	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0
Comatose	0	0	0	0	0	0	0
Salivations	0	0	0	0	0	0	0
<u>Other observations:</u>							

<u>Toxicity Observation Sheet</u> Toxicity study of <i>Sandoricum Koetjape</i> extract							
<u>Animal Number:</u> 4	<u>Treatment Group:</u> Extract		<u>Dose received:</u> 2000 mg/kg		<u>Date and Time the dose was given:</u> March 05, 2026 1:00 pm		
Initial Weight: 32.6 g							
	1st 30 minute s	1 hour	2 H	3H	4H	24 H	Day 14
Weight	32.6 g	32.6 g	32.6 g	32.6 g	32.6 g	32.6 g	36.1 g
Movement	0	0	0	0	0	0	0
Coat Condition	0	0	0	0	0	0	0
Posture	0	0	0	0	0	0	0
Breathing	0	0	0	0	0	0	0
Feces and Urine	0	0	0	0	0	0	0

Attitude	0	0	0	0	0	0	0
Appearance	0	0	0	0	0	0	0
BCS	0	0	0	0	0	0	0
Specific Clinical Signs							
Tremors	0	0	0	0	0	0	0
Circling	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0
Comatose	0	0	0	0	0	0	0
Salivations	0	0	0	0	0	0	0
<i>Other observations:</i>							

Toxicity Observation Sheet							
Toxicity study of <i>Sandoricum Koetjape</i> extract							
<u>Animal Number:</u> 5	<u>Treatment Group:</u> Ointment		<u>Dose received:</u> 5000 mg/kg		<u>Date and Time the dose was given:</u> March 05, 2026 – 1:00 pm		
Initial Weight: 29.2 g							
	1st 30 minute s	1 hour	2 H	3H	4H	24 H	Day 14
Weight	29.2 g	29.2 g	29.2 g	29.2 g	29.2 g	29.2 g	30.3 g
Movement	0	0	0	0	0	0	0
Coat Condition	0	0	0	0	0	0	0
Posture	0	0	0	0	0	0	0
Breathing	0	0	0	0	0	0	0
Feces and Urine	0	0	0	0	0	0	0
Attitude	0	0	0	0	0	0	0
Appearance	0	0	0	0	0	0	0
BCS	0	0	0	0	0	0	0
<u>Specific Clinical Signs</u>							
Tremors	0	0	0	0	0	0	0
Circling	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0
Comatose	0	0	0	0	0	0	0
Salivations	0	0	0	0	0	0	0
<u>Other observations:</u>							

Toxicity Observation Sheet							
Toxicity study of <i>Sandoricum Koetjape</i> extract							
<u>Animal Number:</u> 6	<u>Treatment Group:</u> Extract		<u>Dose received:</u> 2000 mg/kg		<u>Date and Time the dose was given:</u> March 07, 2026 1:00 pm		
Initial Weight: 29 g							
	1st 30 minute s	1 hour	2 H	3H	4H	24 H	Day 14
Weight	29 g	29 g	29 g	29 g	29 g	29 g	31.4 g
Movement	0	0	0	0	0	0	0
Coat Condition	0	0	0	0	0	0	0
Posture	0	0	0	0	0	0	0
Breathing	0	0	0	0	0	0	0
Feces and Urine	0	0	0	0	0	0	0
Attitude	0	0	0	0	0	0	0
Appearance	0	0	0	0	0	0	0
BCS	0	0	0	0	0	0	0
<u>Specific Clinical Signs</u>							
Tremors	0	0	0	0	0	0	0
Circling	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0
Comatose	0	0	0	0	0	0	0
Salivations	0	0	0	0	0	0	0
<u>Other observations:</u>							

Toxicity Observation Sheet Toxicity study of <i>Sandoricum Koetjape</i> extract							
<u>Animal Number:</u> 7	<u>Treatment Group:</u> Ointment		<u>Dose received:</u> 2000 mg/kg		<u>Date and Time the dose was given:</u> March 07, 2026 – 1:00 pm		
Initial Weight: 28.1 g							
	1st 30 minute s	1 hour	2 H	3H	4H	24 H	Day 14
Weight	28.1 g	28.1 g	28.1 g	28.1 g	28.1 g	28.1 g	30.8 g
Movement	0	0	0	0	0	0	0
Coat Condition	0	0	0	0	0	0	0
Posture	0	0	0	0	0	0	0
Breathing	0	0	0	0	0	0	0

Feces and Urine	0	0	0	0	0	0	0
Attitude	0	0	0	0	0	0	0
Appearance	0	0	0	0	0	0	0
BCS	0	0	0	0	0	0	0
Specific Clinical Signs							
Tremors	0	0	0	0	0	0	0
Circling	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0
Comatose	0	0	0	0	0	0	0
Salivations	0	0	0	0	0	0	0
<i>Other observations:</i>							

➤ Necropsy Results

	Liver (weight in g)	Spleen (weight in g)	Spleen length in mm)	Right Kidney (weight in g)	Left Kidney (weight in g)	Any Lesions seen (any organ)	Any other changes observed
M1	1.90 g	0.06 g	12.40 mm	0.23 g	0.25 g	-	-
M2	1.59 g	0.11 g	16.40 mm	0.23 g	0.25 g	-	-
M3	1.48 g	0.06 g	13.80 mm	0.26 g	0.29 g	-	-
M4	2.46 g	0.19 g	20.40 mm	0.27 g	0.28 g	-	-
M5	1.89 g	0.06 g	13.80 mm	0.26 g	0.28 g	-	-
M6	2.02 g	0.12 g	13.80 mm	0.28 g	0.29 g	-	-
M7	1.29 g	0.07 g	16.40 mm	0.21 g	0.20 g	-	-

➤ Wound Healing Observations

• Negative Control Ointment

Mouse ID	Day 0 Area (mm ²)	Day 3 Area (mm ²)	Day 7 Area (mm ²)	Day 14 Area (mm ²)
NCM1	28.27433388	24.74004214	12.64491042	2.13333776
NCM2	28.27433388	21.53549591	8.60305513	0.08246681
NCM3	28.27433388	20.52870189	8.09549157	0.08639380
NCM4	28.27433388	20.38078247	9.81747703	0.37699112
NCM5	28.27433388	24.26813974	13.11614931	0.11044662
NCM6	28.27433388	22.16558703	11.1094570	0.86393798
Mean ± SD	28.27 ± 0	22.27 ± 1.86	10.56 ± 2.07	0.61 ± 0.78

➤ Negative Control Ointment (Wound Reduction)

Mouse ID	Day 3 Reduction (%)	Day 7 Reduction (%)	Day 14 Reduction (%)
NCM1	12.50000002	55.27777781	92.45486112
NCM2	23.83376386	69.57291667	99.70833332
NCM3	27.39456930	71.36805555	99.69444444
NCM4	27.91772723	65.27777782	98.66666666
NCM5	14.16901334	53.61111117	99.60937499
NCM6	21.60527239	60.70833341	96.94444444

➤ *Positive Control Ointment*

Mouse ID	Day 0 Area (mm ²)	Day 3 Area (mm ²)	Day 7 Area (mm ²)	Day 14 Area (mm ²)
PCO1	28.27433388	22.47809541	9.75464518	1.43138815
PCO2	28.27433388	21.64557336	12.88052987	1.31946891
PCO3	28.27433388	21.64557336	8.67079571	0
PCO4	28.27433388	24.97566157	13.83675212	0.47123890
PCO5	28.27433388	22.06183438	9.61916400	0.09424778
PCO6	28.27433388	20.39679028	8.29576809	0
Mean ± SD	28.27 ± 0	22.20 ± 1.48	10.51 ± 2.23	0.55 ± 0.64

➤ *Positive Control Ointment (Wound Reduction)*

Mouse ID	Day 3 Reduction (%)	Day 7 Reduction (%)	Day 14 Reduction (%)
PCO1	20.50000009	65.50000003	94.93750001
PCO2	23.44444452	54.44444448	95.33333335
PCO3	23.44444452	69.33333338	100
PCO4	11.66666675	51.06250008	98.33333333
PCO5	21.97222232	65.97916669	99.66666667
PCO6	27.86111119	70.65972226	100

➤ *5% Concentration Ointment*

Mouse ID	Day 0 Area (mm ²)	Day 3 Area (mm ²)	Day 7 Area (mm ²)	Day 14 Area (mm ²)
1PEO1	28.27433388	20.16902481	10.89347251	0
1PEO2	28.27433388	19.29723285	14.66731068	0.35342917
1PEO3	28.27433388	21.64753685	9.75464518	0
1PEO4	28.27433388	18.62571742	9.20879346	0
1PEO5	28.27433388	23.97427891	10.59502121	0
1PEO6	28.27433388	23.31061746	11.73384855	0.07068583
Mean ± SD	28.27 ± 0	21.17 ± 2.18	11.14 ± 1.86	0.07 ± 0.14

➤ *5% Concentration Ointment (Wound Reduction)*

Mouse ID	Day 3 Reduction (%)	Day 7 Reduction (%)	Day 14 Reduction (%)
1PEO1	28.66666675	61.47222228	100
1PEO2	31.75000008	48.12500007	98.75000001
1PEO3	23.43750010	65.50000003	100
1PEO4	34.12500008	67.43055557	100
1PEO5	15.20833342	62.52777783	100
1PEO6	17.55555565	58.50000004	99.75000002

➤ *10% Concentration Ointment*

Mouse ID	Day 0 Area (mm ²)	Day 3 Area (mm ²)	Day 7 Area (mm ²)	Day 14 Area (mm ²)
2PEO1	28.27433388	19.72134786	9.89601685	0
2PEO2	28.27433388	23.54230992	11.87522022	0
2PEO3	28.27433388	20.73451149	8.65901474	0
2PEO4	28.27433388	21.03296279	10.74424686	0
2PEO5	28.27433388	20.81305131	9.88030888	0
2PEO6	28.27433388	17.86780820	10.30835088	0
Mean ± SD	28.27 ± 0	20.62 ± 1.77	10.23 ± 1.09	0 ± 0

➤ 10% Concentration Ointment (Wound Reduction)

Mouse ID	Day 3 Reduction (%)	Day 7 Reduction (%)	Day 14 Reduction (%)
2PEO1	30.25000008	65.0000	100
2PEO2	16.73611120	58.0000	100
2PEO3	26.66666494	69.37500004	100
2PEO4	25.61111120	62.0000	100
2PEO5	26.38888895	65.05555561	100
2PEO6	36.80555561	63.54166672	100