

Wound Burn Healing Property of Puting Anghel (*Wrightia antidysenterica*) Leaves Extract as Topical Cream in Wistar Albino Rats

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Abstract

Burn wound injuries are prevalent global health issues that frequently result in pain, infection, scarring, and even death. In the Philippines, the UP-PGH ATR Burn Center reports that most second-degree burn patients are children (50%) and working-age adults (25%) due to poor supervision and work accidents. Effective treatments are essential to promote healing, reduce complications, and minimize scarring. Puting Anghel (*Wrightia antidysenterica*) leaves contain flavonoids, tannins, phenols, and alkaloids, suggesting wound healing potential when formulated into a cream. Patch and Scratch Tests on three guinea pigs confirmed its safety, showing no signs of irritation. The cream's effectiveness was evaluated using five rat groups: three received different concentrations of Puting Anghel cream, one received Aloe Vera gel (positive control), and one received cream base (negative control). Histopathological analysis and epithelialization time were assessed. ANOVA and post-hoc analyses showed that the 75% concentration of Puting Anghel cream had significantly better re-epithelialization, neovascularization, granulation tissue formation, fibroblast maturation, and lower inflammation compared to the cream base ($p < 0.05$). No significant difference was found between the 75% Puting Anghel cream and Aloe Vera gel, indicating comparable healing effects. The study concludes that 75% Puting Anghel cream is a safe and effective wound treatment.

Keywords: Second-degree burn, Puting Anghel, *Wrightia antidysenterica*, Wound healing

1. Introduction

Burn injuries, particularly second-degree burns, are among the most painful and complex skin traumas, affecting both the epidermis and dermis. These wounds often result in blistering, compromised skin integrity, and increased susceptibility to infections. Globally, burn injuries account for approximately 180,000 deaths each year, with the highest burden in low- and middle-income countries where access to advanced wound care is limited (WHO, 2020). In the Philippines, burns remain a common cause of emergency visits and hospital admissions, especially among children and individuals working in hazardous environments like construction and electrical services (Tan et al., 2017). Although conventional treatments such as silver sulfadiazine and antibiotic-based creams are available, they are often expensive, may cause adverse effects like skin irritation, and can contribute to antimicrobial resistance with prolonged use (Ivanko et al., 2024). These limitations have prompted increasing interest in safer, more affordable alternatives, particularly those derived from medicinal plants.

Wrightia antidysenterica locally known in the Philippines as Puting Anghel or White Angel, is a small ornamental shrub belonging to the Apocynaceae family. Native to South and Southeast Asia, it is characterized by its glossy green leaves and star-shaped white flowers. Traditionally used in Ayurvedic, Unani, and Filipino herbal medicine, the plant has been applied topically and orally to manage a variety of conditions including eczema, psoriasis, fungal infections, and chronic wounds (Stuart, 2024). Ethnobotanical records highlight its use for treating dysentery, skin ulcers, and inflammatory disorders.

The therapeutic potential of *W. antidysenterica* is attributed to its rich phytochemical composition, which includes flavonoids, phenolic acids, tannins, alkaloids, and iridoids. These compounds have demonstrated notable antimicrobial, anti-inflammatory, and antioxidant properties that are key mechanisms in the wound healing process. Flavonoids and tannins contribute to tissue repair by enhancing collagen synthesis, reducing oxidative stress, and inhibiting bacterial colonization, while phenols provide anti-inflammatory support by modulating cytokine activity (Chauhan et al., 2020; Kovac et al., 2024). These properties suggest that *W. antidysenterica* has the potential to serve as an effective natural remedy for skin injuries, particularly burns.

Topical application is an effective mode of delivery for herbal wound therapies, as it allows for localized action at the injury site while minimizing systemic effects. Cream formulations, in particular, offer several advantages: they are easy to apply, non-greasy, promote moisture retention, and enhance patient compliance (Cherney et al., 2022; Ohnstedt et al., 2019). Incorporating *W. antidysenterica* extract into a topical cream provides a promising approach to harness its bioactive compounds for therapeutic use in burn wound healing.

Therefore, this study aims to develop a topical cream formulation using *Wrightia antidysenterica* leaf extract and evaluate its wound healing efficacy on experimentally induced second-degree burns in Wistar albino rats. The study also seeks to assess the cream's anti-inflammatory, antimicrobial, and antioxidant effects, comparing its performance with that of standard topical treatments. Ultimately, this research hopes to provide scientific support for the plant's traditional use and contribute to the development of plant-based therapies that are accessible, affordable, and effective for burn wound management.

2. Objectives

The primary objective of this study is to evaluate the wound burn healing property of Puting Anghel (*Wrightia antidysenterica*) when formulated as a topical cream. It aims to determine the most effective concentration among 25%, 50%, and 75% in promoting wound healing in Wistar albino rats. The healing effect will be assessed through epithelialization time and histopathological examination. Key histological indicators include re-epithelialization, neovascularization, granulation tissue development, fibroblast maturation, and the presence of acute inflammation. The study also seeks to identify any significant differences in wound healing outcomes between the most effective concentration, a positive control group using silver sulfadiazine, and a negative control group using base cream. Patch and Scratch tests will be conducted to evaluate skin irritation and determine the Primary Irritation Index for each concentration. Additionally, the physicochemical and organoleptic properties of the cream formulations will be assessed, including color, odor, texture, and pH. The formulations will be monitored for stability and consistency throughout the study. Ultimately, the study aims to provide scientific evidence supporting the use of *Wrightia antidysenterica* as a natural, plant-based wound healing agent.

3. Materials and Methods

The materials and methodology used to determine the Puting Anghel (*Wrightia antidysenterica*) extract's wound burn healing qualities are described in this section. This section has covered the study's methodology, test animals, data collection instruments, research design, duration, and location.

Research Design

This study employed an experimental research design to evaluate the wound burn healing property of Puting Anghel (*Wrightia antidysenterica*) leaves extract formulated as a topical cream in Wistar albino rats. An experimental design was appropriate for this type of study because it allowed controlled manipulation and observation of the effects of the formulated cream on burn wound healing. The researchers were able to prove the effects of the bioactive components by using a controlled animal model to reduce variables and evaluate its therapeutic efficacy in a scientifically accurate way.

The plant sample was collected, then was subjected to Soxhlet extraction in order to obtain the active compounds from the plant material. After the extraction, the solvent was removed using a rotary evaporator (Rotavap) to concentrate the extract and remove the extracting solvent, followed by phytochemical testing to identify the bioactive constituents present in Puting Anghel. A topical cream was then formulated using the extract. The Wistar albino rats were divided into five groups: the first three groups received the treatment groups with varying concentrations (25%, 50%, & 75%), the Positive Control Group as the fourth, and the Negative Control Group as the fifth.

Burn wounds were inflicted on all the Wistar albino rats, and their healing responses were assessed by measuring wound contraction and healing time, and observing histopathological changes.

Collection of Plant Material

The leaves of the Puting Angel (*Wrightia antidysenterica*), which were collected from the yard of one of the researchers residing at Barangay Central East, Bauang, La Union, were utilized as the plant material in this study. The locale was specifically chosen due to the availability and well-maintained quality of the plant sample. The collected plant samples were

sent to Don Mariano Marcos Memorial State University - North La Union Campus for plant authentication.

Preparation of Puting Angel Leaves Extract

The leaves of Puting Angel were collected and thoroughly washed to eliminate any contaminants before the extraction process. A sufficient amount of the leaves was dried in the oven at 40 degrees Celsius for 48 hours and pulverized into coarse powder using a mortar and pestle, then sieved using Sieve Number 10 to produce 1000 grams, which were placed in the extraction chamber of a Soxhlet apparatus. A round-bottom flask containing ethanol was attached to the apparatus. The mixture was heated until the ethanol reached its boiling point, allowing the vapor to rise into the condenser, where it cooled and dripped back into the chamber to dissolve the active compounds from the leaves. Once the extraction was complete, the heat was turned off, and the apparatus was allowed to cool before the round-bottom flask was detached. After the Soxhlet extraction process, the extract was subjected to a rotary evaporation to separate the remaining extracting solvent from the extract. The extract was placed in a flask, which was partially submerged in water at a temperature of 40 degrees Celsius, with the rotation set to 280 rpm. This allowed the extracting solvent to evaporate, and the plant extract was collected in the large round-bottomed flask reservoir. The rotary evaporation process was carefully monitored and stopped once no solvent was dripping from the condenser. The resulting extract was then subjected to phytochemical testing and testing for the absence of ethanol.

Testing Procedure for the Absence of Ethanol

The resulting Puting Angel leaves extract was subjected to Iodoform Test after the Rotary Evaporation extraction to check if there was still the presence of ethanol which could interfere with the results (*Appendix G*).

Phytochemical Analysis of the Plant Extract

Phytochemical screening was performed on Puting Angel leaves extract to identify and assess the presence of active constituents that contributed to its therapeutic properties.

This screening confirmed the presence of key phytochemicals such as alkaloids, phenols, tannins, and flavonoids, which were responsible for its potential wound healing effects (*Appendix H*).

Formulation of the Cream Base

A water-in-oil (W/O) emulsion-based cream (semisolid formulation) provided a rich, moisturizing texture that locked in moisture and reduced transepidermal water loss. It enhanced the stability and efficacy of oil-soluble active ingredients while creating a protective barrier, making it effective for dry or irritated skin. To prepare a W/O cream, the oil and emulsifier, as well as the water phase, were heated separately. The water phase was mixed with the oil phase while stirring, and the mixture was cooled to room temperature (*Appendix J*).

Formulation of the Puting Angel Topical Cream

After the formulation of the cream base, a topical cream of Puting Angel was prepared to assess its effectiveness, safety, and wound healing potential and to provide a profile of its organoleptic (color, odor, texture) properties and pH. To prepare the topical creams, the different

concentrations of Puting Angel leaf extract were mixed with the cream base until they became uniformly incorporated (*Appendix K*).

Acclimatization of Test Animals

The male guinea pigs, with an average weight ranging from 300g to 420g, and the male Wistar albino rats, with an average weight ranging from 200g to 250g, were acclimatized in the animal house of Lorma Colleges for 14 days to allow them to adjust to their new environment. During this period, the guinea pigs and rats were housed in a clean, dry environment at room temperature and were fed pellets and provided with clean water. All rats and guinea pigs were individually housed in a cage which were labeled with stickers according to the treatment groups and/or tests to which they were subjected. The housing and beddings of the rats and guinea pigs were cleaned and replaced twice a week. The rats and guinea pigs were supplied with food and water twice a day, in the morning and in the afternoon. After the acclimatization period, the 15 rats were divided into five experimental groups, while an additional 3 guinea pigs were used for the patch and scratch test. The rats were then grouped as per assigned treatment.

The first group was treated with a 75% concentration of Puting Angel cream, second group with a 50% concentration, and the third group with a 25% concentration. The fourth group was treated with a commercially available product, Aloe vera gel, as the positive control group. Lastly, the fifth group, the negative control group received the cream base.

The three (3) guinea pigs were subjected to the patch and scratch tests using the three different concentrations of the Puting Angel formulated cream to determine its safety.

Patch and Scratch Tests

A total of three (3) guinea pigs were used for the Patch and Scratch Tests, with each guinea pig assigned to one of the three concentrations of the Puting Angel topical cream. The left side of each guinea pig was used for the Patch Test, and the right side was used for the Scratch Test, ensuring each concentration was tested on a single animal.

In the Patch Test, the skin on the left side lateral to the spinal groove of each guinea pig was shaved and cleaned. The Puting Angel topical cream with the assigned concentration was applied to this area. Sterilized gauze was placed over the applied cream, and surgical tape secured the gauze in place. The guinea pigs were left undisturbed for 24 hours. After the 24-hour period, the gauze was carefully removed, and any skin reactions were assessed using a standardized scoring system.

For the Scratch Test, the skin on the right side lateral to the spinal groove was gently abraded using a 20-gauge hypodermic needle to create five to seven light scratches. After the abrasion, the Puting Angel topical cream at the same assigned concentration was applied to the scratched area. Skin reactions were observed and recorded immediately after the application, similar to the Patch Test, and were scored using the same standardized system.

Table 1.*Evaluation of Skin Reaction through Erythema and Edema Formation*

Erythema Formation	Score	Edema Formation
No erythema	0	No edema
Very slight erythema	1	Very slight edema
Well-defined erythema	2	Well-defined edema
Moderate to severe erythema	3	Moderate to severe edema
Severe erythema	4	Severe edema

Legend: 0 - No edema / No erythema, 1 - Very slight edema / Very slight erythema, 2 - Well-defined edema / Well-defined erythema, 3 - Moderate to severe edema / Moderate to severe erythema, 4 - Severe edema / Severe erythema

The scores from both the Patch and Scratch Tests were compiled and compared to assess the safety and potential irritancy of the Puting Angel topical cream. The safety level was determined using the Primary Irritation Index (PII), calculated as follows:

Primary Irritation Index = $\frac{1}{2}$ (Average of the Patch Test + Average of the Scratch Test)

Table 2.*Primary Irritation Index and its Classification*

Primary Irritation Index	Classification
0	No irritation
> 0 - 2.0	Slight irritation
2.1 - 5.0	Moderate irritation
> 5.0	Severe irritation

Source: Shara, M., Yasmin, T., Kincaid, A., Limpach, A., Bartz, J., Brenneman, K., Chatterjee, A. & Bagchi, M., Stohs, S. & Bagchi, D. (2005). Safety and toxicological evaluation of a novel niacin-bound chromium(III) complex.

Infliction of Burn Wound

To inflict a full-thickness second-degree burn wound on the Wistar albino rats, the hair on their backs was shaved, and the skin was disinfected using 70% isopropyl alcohol, then allowed to dry for 3 minutes. Afterward, the rats were anesthetized through inhalation of chloroform to ensure they remained still and free of discomfort during the procedure.

A cylindrical steel rod with a diameter of 1 cm was heated in boiling water at 100°C for 10 seconds and was then immediately pressed gently against the disinfected skin of the rats for exactly 10 seconds. After this time, the heated rod was withdrawn, resulting in a controlled second-degree burn on the skin. This procedure was repeated for all rats involved in the experiment (Cai et al., 2014).

This method of 2nd degree burn infliction was based on a procedure in a previous similar study entitled "Wound contraction effect of Bermuda grass (*Cynodon dactylon*) topical cream on induce second-degree burn in male Albino mice", in which they also used pre-heated Cylindrical stainless steel rods to inflict burn injury for 10 seconds to successfully inflict a second degree burn (Rallos et al., 2020).

Administration of Treatment

Each Wistar albino rat selected for the study received its first topical treatment one day after the burn injury. A pathologist confirmed that the wounds were second-degree burns, exposing the dermis layer of the skin. The rats were then assigned to the following groups:

Group I (Treatment 1): This group was treated with the formulated Puting Anghel cream with the concentration of 75.

Group II (Treatment 2): This group was treated with the formulated Puting Anghel cream with the concentration of 50%.

Group III (Treatment 3): This group was treated with the formulated Puting Anghel cream with the concentration of 25%.

Group IV (Positive Control Group): This group was treated with Aloe vera gel.

Group V (Negative Control Group): This group was treated with cream base only.

The treatments were administered twice a day for a duration of fourteen days. The rats were monitored regularly throughout the study to observe wound healing and assess the effectiveness of each treatment.

Determination of Burn Wound Healing Response

The burn wound healing process was evaluated through the following criteria:

1. Epithelialization Time

The burn wound healing process was recorded by the researchers at specific intervals (1st, 3rd, 6th, 9th, 12th, and 14th day) following the application of the treatment. The epithelialization time was assessed by tracking the number of days it took for the scab to detach naturally, revealing healed skin underneath without any exposed wound.

2. Histopathological Examination

Upon completion of the treatment period, tissue samples were collected by a licensed veterinarian at Seika Veterinary in Naguillian, La Union from the burn wound sites for histopathological analysis. These samples were processed in a pathology laboratory in Pines City Doctor's Hospital in Baguio City, Benguet, and the resulting slides were examined under a

microscope. The examination focused on tissue regeneration indicators such as collagen production, fibroblast activity, and overall tissue structure.

Evaluation of Topical Cream

The formulated topical cream was observed and evaluated based on its organoleptic properties, such as color, odor, and texture. Its pH level was also evaluated. Organoleptic evaluation was conducted by three (3) selected evaluators: a faculty instructor from the College of Pharmacy, a second-year Pharmacy student, and a student from a different college department at Lorma Colleges - Campus for Health Sciences. The evaluators assessed the product by responding to a structured questionnaire (Appendix R) designed to characterize its sensory attributes designed to characterize its sensory attributes. Additionally, the pH of the topical cream was determined using a calibrated pH strip.

4. Results

Table 3.

Results of Patch Test to Determine the Primary Irritation Index of Puting Anghel Leaves

Guinea Pigs	After 24 hours	Average Scores	Interpretation
1 (75% cream)	0	0	No erythema
2 (50% cream)	0	0	No erythema
3 (25% cream)	0	0	No erythema

Legend: 0 - No erythema, 1 - Very slight erythema, 2 - Well-defined erythema, 3 - Moderate to severe erythema, 4 - Severe erythema

Table 4.

Results of Scratch Test to Determine the Primary Irritation Index of Puting Anghel Leaves

Guinea Pigs	After 24 hours	Average Scores	Interpretation
1 (75% cream)	0	0	No edema
2 (50% cream)	0	0	No edema
3 (25% cream)	0	0	No edema

Legend: 0 - No edema, 1 - Very slight edema, 2 - Well-defined edema, 3 - Moderate to severe edema, 4 - Severe edema

Table 5.*Primary Irritation Index (PII) Computation*

Patch Test	
Formula	$\frac{\text{Total Score}}{\text{Total Score for Erythema}} = \text{Average for 24 hours of reading}$
Solution	$\frac{0}{3} = 0$
Result	0

Scratch Test	
Formula	$\frac{\text{Total Score}}{\text{Total Score of Edema}} = \text{Average for 24 hours reading}$
Solution	$\frac{0}{3} = 0$
Result	0

Primary Irritation Index (PII)	
Formula	Primary Irritation Index = $\frac{1}{2}$ (Average of the Patch + Average of the Scratch)
Solution	Primary Irritation Index = $\frac{1}{2} (0+0) = 0$
Result	0

Table 6.*Histological Examination Results in terms of the given parameters that indicate Wound Healing*

		Re-epithelialization	Neovascularization	Granulation Tissue Amount	Granulation Tissue Fibroblast Maturation	Acute Inflammation
	No.1	3	2	2	3	1
TG #1	No.2	2	2	2	2	1
(75%)	No.3	3	2	2	3	1
Mean		2.7	2	2	2.7	1
	No.1	2	2	2	2	1
TG #2	No.2	2	2	2	2	1
(50%)	No.3	2	2	2	3	1
Mean		2	2	2	2.3	1
	No.1	2	2	2	2	2
TG #3	No.2	2	2	2	2	2
(25%)	No.3	2	2	2	2	2
Mean		2	2	2	2	2

*Legend: TG – Treatment Group***Table 7.***Epithelialization Time (days) of Burn Wound* *Legend: TA – Test Animal; TG – Treatment Group*

	TA 1	TA 2	TA 3	Mean
	Number of days of Epithelialization			
TG #1	13 days	12 days	13 days	12.7 days
(75%)				
TG #2	13 days	14 days	13 days	13.3 days
(50%)				
TG #3	14 days	14 days	14 days	14 days
(25%)				

Table 8.

Analysis of Variance (ANOVA) of Results of Wound Healing Response in terms of Re-Epithelialization, Neovascularization, Granulation Tissue Amount, and Granulation Tissue Fibroblast Maturation

ANOVA Table					
Source	SS	df	MS	f	p-value
Treatment	1.851852	2	0.9259259	5.77	.0244
Error	1.444444	9	0.9259259		
Total	3.296296	11			

Table 9.

Post hoc Analysis of the Wound Healing Response in terms of Re-Epithelialization, Neovascularization, Granulation Tissue Amount, and Granulation Tissue Fibroblast Maturation

	NC	TG1	PC
	1.500000	2.33333	2.33333
NG	1.50000		
TG1	2.33333	2.94	
PC	2.33333	2.94	0.00

Legend: Critical Values for experiment-wise error rate = 0.05 = 2.79

NG – Negative Group; TG1 – Treatment Group 1; PC – Positive Control

Table 10.

Analysis of Variance (ANOVA) of Results of Wound Healing Response in terms of Epithelialization Time

ANOVA Table					
Source	SS	df	MS	f	p-value
Treatment	2.00	2	1.000	270215977642230 00.00	1.37E-48
Error	0.00	6	0.000		
Total	2.00	8			

Table 11.*Post hoc Analysis of the Wound Healing Response in terms of Acute Inflammation*

		TG1	PC	NC
		1.0	1.0	2.0
TG #1 (75% cream)	1.0			
PC	1.0	1.0000		
NC	2.0	1.01E-48	1.01E-48	

Legend: TG #1 (75% cream) – Treatment Group #1 (75% cream); PC – Positive Control;
NC – Negative Control

Table 12.*Analysis of Variance (ANOVA) of Result of Wound Healing Responses in terms of Epithelialization Time***ANOVA table**

Source	SS	df	MS	F	p-value
Treatment	4.67	2	2.333	4.20	.0723
Error	3.33	6	0.556		
Total	8.00	8			

Table 13.*Characteristics of the Prepared Topical Cream*

Characteristics	Evaluation
A. Organoleptic Properties	
Color	Pale Brown
Odor	Moderately Aromatic
Texture	Smooth and Creamy
B. pH	4-6

5. Discussion

Primary Irritation Index of the Puting Anghel Leaves Extract Topical Cream

Table 3 shows the erythema results for the Patch Test. The formation of erythema on the skin of the guinea pigs after the patch test was interpreted through the standardized scoring system (Table 1). Upon computation, the average erythema score was zero (0), indicating no persistent erythema was found in any of the three guinea pigs tested for the different concentrations of the Puting Anghel cream.

According to Bhattacharyya et al. (2016), *W. antidysenterica* demonstrated significant effectiveness in alleviating symptoms such as scaling and itching, which are commonly associated with skin irritation and inflammation. These symptoms often lead to erythema, or redness of the skin, due to increased blood flow and inflammatory response in the affected area. The reduction in scaling and itching observed in the study suggests that *W. antidysenterica* possesses anti-inflammatory and soothing properties that help prevent the development of erythema. This supports the findings presented in Table 3, where no persistent erythema was observed following the application of the Puting Anghel topical cream, indicating the formulation's effectiveness in minimizing skin irritation during the wound healing process.

Table 4 shows the edema results for the Patch Test. The formation of edema on the skin of the guinea pigs after the scratch test was interpreted through the standardized scoring system (Table 1). Upon computation, the average erythema score was zero (0), indicating no persistent erythema was found in any of the three guinea pigs tested for the different concentrations of the Puting Anghel cream (Appendix L).

The absence of edema was attributed to the presence of natural compounds in the Puting Anghel plant, such as flavonoids, tannins, phenols, and alkaloids. These bioactive constituents, aside from their established wound healing properties, protected skin cells and supported capillary stability. Tannins exhibited a mild astringent effect that tightened tissues and prevented fluid leakage, thereby reducing the occurrence of edema (Ashok & Upadhyaya, 2012). Flavonoids and phenols protected cell membranes from oxidative stress, which could have otherwise contributed to edema through tissue damage and fluid buildup (Guevara-Vasquez et al., 2021; Rajashekar, 2023). Alkaloids promote skin repair and prevent tissue breakdown (Budiawan et al., 2023). These combined mechanisms effectively prevented the formation of edema, confirming that the cream was non-irritating and safe for topical application.

The Patch and Scratch test findings, as shown in Tables 3 and 4, indicated that there were no signs of erythema or edema after 24 hours. Based on the skin irritation evaluation table, the average score for both erythema and edema was zero (0) in the Patch and Scratch Tests. These findings confirmed that the topical cream did not cause skin irritation and was safe for further testing.

Most Effective Concentration of the Formulated Puting Angel Topical Cream in Terms of:

A. Histopathological Examination Results

Table 6 shows the histopathological examination results in terms of the given parameters that indicate wound healing, which consists of re-epithelialization,

neovascularization, granular tissue amount, granular tissue fibroblast maturation, and acute inflammation. Each of these parameters was evaluated through a scoring system for histopathological examination on skin wound (Appendix M).

Re-epithelialization involves the formation of new epithelium and skin appendages by activating the proliferation, migration, and differentiation of keratinocytes and reconstituting the protection of the underlying dermal structures to prevent infection and excessive moisture loss (Chen et al., 2015). The keratinocytes become activated, and the activation process is achieved by the expression of several cytokines and growth factors. The activated phenotype is marked by changes in the cytoskeleton network and cell surface receptors essential for re-epithelialization, namely, expression of K6 and K16, allowing keratinocytes to migrate into the wound to fill the defect (Pastar et al., 2015). Treatment Group 1 exhibited a mean of 2.7, while Treatment Groups 2 and 3 both showed a mean of 2. So, the mean score of 2.7 indicates a higher level of re-epithelialization and suggests that the 75% concentration was more effective in promoting tissue regeneration, which indicates a potential dose-dependent effect. Supporting this interpretation, studies demonstrated that certain concentrations of bioactive extracts can enhance keratinocyte proliferation, migration, and tissue regeneration, which are key processes in re-epithelialization. In the *in vivo* wound healing study with Sprague-Dawley rats, a 1.25% concentration of C-phycoerythrin (C-PC) showed superior effects on tissue regeneration by day 7 compared to other preparations (Sevimli Gur et al., 2013).

Neovascularization is the natural formation of new blood vessels that serve as collateral circulation in response to poor local perfusion (Szwedowski et al., 2021). The neovascularization process is crucial in the connective tissue healing process. During the regeneration, in the formation phase, intensive neovascularization is observed. Effective wound healing requires the vascularization of the newly formed tissue. As granulation tissue forms, the number of blood vessels in the dermis increases. A significant increase in the density of blood vessels compared to uninjured skin is commonly observed during the proliferative phase of healing as a result of angiogenesis (Johnson & Wilgus, 2014). In the neovascularization of wounds in the test animals, all treatment groups showed a neovascularization score of 2, indicating the presence of 6–10 vessels per high-power microscopic field (HMF). Since all treatment groups had the same average mean of 2, it suggests that this level of neovascularization is typical of the proliferative phase of wound healing, where new blood vessel formation is essential (Moreira & Marques, 2022). The interpretation is based on a semi-quantitative scoring system for neovascularization, where the number of new blood vessels per high-power microscopic field (HMF) is counted and assigned a score. A mean score of 2 means that on average, there were about 6 to 10 new blood vessels seen in each high-power microscopic field. This shows that the treatment regardless of the concentration was able to support a good level of new blood vessel formation. Since all groups had the same score, it suggests that

even the lowest concentration was already enough to trigger this response and increasing the dose didn't lead to more blood vessels being formed. Therefore, all concentrations of the Puting Anghel topical cream demonstrated a similar wound healing response based on neovascularization, despite having different concentrations.

Granulation tissue is a transitional replacement for the normal dermis, which eventually matures into a scar during the remodeling phase of healing. It is characterized from the unwounded dermis by an extremely dense network of blood vessels and capillaries, elevated cellular density of fibroblasts and macrophages and randomly organized collagen fibers. It also has an elevated metabolic rate compared to the normal dermis, reflecting the activity required for cellular migration, division, and protein synthesis (Schultz et al., 2011). In the granulation tissue amount in the wounds of the test animals, all test animals in all treatment groups had a granulation tissue amount scoring of 2, which suggested a moderate amount of granulation tissue involving 11-20 vessel formation in 1 high-power microscopic field (HMF). Since all treatment groups had the same average mean of 2, it suggests that there is an active tissue repair and matrix deposition that supports keratinocyte migration and wound contraction (Alhaji & Goyal, 2022). Therefore, all concentrations of the Puting Anghel topical cream demonstrated a similar wound healing response, based on the amount of granulation tissue regardless of concentration.

In granular tissue fibroblast maturation during the wound healing process, fibroblasts can transform into myofibroblasts, a conversion that underlies their ability to produce fibrotic and wound contraction and closure. This healing-related differentiation of fibroblasts is triggered by mechanical signaling, cytokines, and growth factors, with transforming growth factor-beta (TGF β) serving as a key mediator (Cialdai et al., 2022). Based on the computed average, Treatment Group 1 had a mean of 2.7, followed by Treatment Group 2 with a mean of 2.3, and Treatment Group 3 with a mean of 2. Supporting this interpretation, studies from an in vivo wound healing study with Sprague-Dawley rat showed that the 1.25% concentration of C-phycoerythrin (C-PC) resulted in more mature and denser granulation tissue, with higher number of fibroblasts and capillaries compared to the 0.25% C-phycoerythrin solution. (Sevimli Gur et al., 2013). So the mean score of 2.7 indicates a higher level of maturation and suggests that the 75% concentration was more effective in promoting granulation tissue fibroblast maturation, which indicates a potential dose-dependent effect.

Acute inflammation is the process by which the body responds to burn injuries with the initial release of inflammatory mediators, specifically cytokines that trigger both local and systemic inflammatory reactions (Noorbakhsh et al., 2021). Both of the average means for the 75% and 50% are 1, while 25% is 2. The interpretation is based on a semi-quantitative scoring system for acute inflammation where mixed inflammation concentrating around vessels is observed and assigned a score. The 25% concentration group has an inflammation score of 2, higher than the 75% and 50% groups, which both

have a score of 1. A higher inflammation score indicates more acute inflammation, which is not necessarily beneficial. The mean score of 1 indicates a minimal amount of inflammation, suggesting that the 75% concentration was more effective in reducing inflammation, which indicates a potential dose-dependent effect. Supporting this interpretation, studies from the in vivo wound healing model with Sprague-Dawley rats demonstrated that the 1.25% concentration of C-phycoerythrin (C-PC) led to markedly reduced acute inflammation at the wound site compared to the 0.25% C-PC and control groups. Histological examination revealed that wounds treated with 1.25% C-PC exhibited less inflammatory cell infiltration, indicating a more controlled and resolved inflammatory response. In contrast, both the control and 0.25% C-PC groups showed more pronounced acute inflammation, with higher levels of inflammatory cell presence in the wound tissue (Sevimli Gur et al., 2013).

The mean was computed through the summation of the scores of groups in each criterion. It could be seen that Treatment Group 1 had a higher average mean for re-epithelialization and granulation tissue fibroblast maturation compared to Treatment 2 and 3. In the acute inflammation, Treatment 1 and 2 exhibited scattered, small amounts of mixed inflammation compared to Treatment Group 3. However, in terms of neovascularization and granulation tissue amount, it can be seen that all treatment groups' average mean are the same. Based on the results of re-epithelialization, granulation tissue fibroblast maturation, and acute inflammation, where the 75% concentration was superior to the two other concentrations, it can be concluded that 75% is the most effective concentration.

Wrightia antidysenterica (Puting Anghel) contains several bioactive constituents, such as flavonoids, tannins, phenols, and alkaloids, which are known for their roles in promoting wound healing. Flavonoids and phenols possess antioxidant and anti-inflammatory properties that help reduce oxidative stress and inflammation at the wound site (Guevara-Vasquez et al., 2021; Vitale et al., 2022). Tannins contribute to epithelialization and the prevention of infection, while alkaloids aid in tissue regeneration (Chokotho et al., 2020; Budiawan et al.). According to concentration, it is expected that the 75% formulated cream will contain the highest amount of constituents. Hence, it has the most notable effect on the identified parameters. However, it is also noteworthy that there were no significant differences in its effect on neovascularization and the amount of granular tissue amount.

B. Epithelialization time (days)

In Table 7, the mean epithelialization times for each treatment group were calculated by summing the number of days it took for the scab to detach from the wound site in each subject naturally and then dividing by the number of subjects in the group.

The results showed that Treatment Group 1, which received the 75% Puting Anghel extract topical cream, had the shortest mean epithelialization time at 12.7 days.

Treatment Group 2 (50% concentration) had a mean of 13.3 days, and Treatment Group 3 (25% concentration) exhibited the longest healing time at 14 days.

The observed trend suggested a concentration-dependent relationship between Puting Angel extract and wound healing, with the 75% concentration exhibiting the most rapid epithelialization. This enhanced healing may be attributed to the synergistic action of various bioactive compounds present in *Wrightia antidysenterica*. Libag et al. (2019) reported the presence of flavonoids, tannins, and alkaloids in this plant, all of which were known for their anti-inflammatory, antioxidant, and antimicrobial properties. These compounds likely contributed to wound healing by modulating key cellular processes such as angiogenesis, collagen synthesis, and extracellular matrix remodeling. Furthermore, *Wrightia antidysenterica* had a history of traditional use in wound care and treatment of skin ailments, suggesting its inherent therapeutic potential (Fern, 2025). Therefore, the faster epithelialization observed with the 75% Puting Angel extract topical cream may have resulted from the combined action of these phytochemicals, leading to an accelerated wound healing response.

The results from both the Histopathological Examination and Epithelialization Time (days) demonstrate that Treatment Group 1 (75% Puting Anghel topical cream) was the most effective for wound healing. Histopathologically, Group 1 had the highest scores for re-epithelialization and granulation tissue fibroblast maturation, indicating more complete and mature healing compared to Groups 2 and 3. Most of the animals in Group 1 showed "complete but immature" re-epithelialization, and one achieved full maturation. Additionally, Group 1 showed lower levels of acute inflammation, suggesting a more efficient healing process. In terms of epithelialization time, Treatment Group 1 had the shortest mean healing time of 12.7 days, compared to 13.3 days for Group 2 and 14 days for Group 3. The faster healing observed in Group 1 was likely due to the higher concentration of bioactive compounds in the 75% Puting Anghel cream. These compounds, such as flavonoids, tannins, and alkaloids, are known for their anti-inflammatory and wound healing properties. Therefore, both the histopathological results and epithelialization times support the superior efficacy of the 75% concentration of Puting Anghel topical cream.

Difference of the Wound Healing Response of 75% Puting Anghel Topical Cream, Positive Control Group, and Negative Control Group in terms of:

A. Histopathological Examination

a.1. Re-Epithelialization, Neovascularization, Granulation Tissue Amount, and Granulation Tissue Fibroblast Maturation

In Table 8, the mean values were calculated by first obtaining the average score of each group (75% Puting Anghel topical cream, positive control, and negative control) for each histopathological criterion—namely, re-epithelialization, neovascularization, granulation tissue amount, and granulation tissue fibroblast maturation. After computing the individual group means for each of these four criteria, the overall mean per group was determined by summing these values and dividing the total by four. This process provided a single representative mean score for each group, reflecting their overall wound healing response based on histopathological assessment.

As shown in Table 8, the resulting p-value of 0.0244 was less than the significance level of 0.05, indicating that there was a statistically significant difference in the wound healing responses among the three groups: the 75% Puting Anghel topical cream group, the positive control group (Aloe vera gel), and the negative control group (cream base only). to the Post Hoc Analysis results and the critical value of 2.79, set for an experimentwise error rate of 0.05, the comparisons between the groups provide meaningful insights into the effectiveness of the treatments. When comparing the 75% Puting Anghel treatment group with the positive control group (Aloe vera gel), no significant difference was observed, as both groups had a computed value of 2.94. However, both the 75% Puting Anghel group and the positive control group showed a significant difference when compared to the negative control group, with a computed value of 0.00, which is lower than the critical value of 2.79. Since the computed value of 2.94 is greater than the critical value, this indicates that the wound healing responses in the 75% Puting Anghel and positive control groups are significantly better than in the negative control group.

According to Chauhan et al. (2020), the presence of active compounds such as flavonoids, tannins, and alkaloids—which have been shown to possess anti-inflammatory, antimicrobial, and antioxidant properties—suggests that *W. antidysenterica* may promote healing and prevent infection in burn wounds. Flavonoids can reduce inflammatory responses and tissue damage, thus facilitating more efficient healing. The hydroxyl group in flavonoids, particularly at critical positions within their chemical structure, enhance their antioxidant, anti-inflammatory, and antibacterial activities, making them essential for skin health (Guevara-Vasquez et al., 2021). Recent research has illuminated the role of tannins in enhancing wound healing by forming protective layers over damaged tissue and precipitating toxic substances, thereby promoting epithelialization and reducing scar formation. Tannins also alleviate pain and prevent secondary infections, which are critical aspects of effective wound management (Chokotho et al., 2020).

Phenolic compounds facilitate cell proliferation and angiogenesis, thus enhancing tissue regeneration. (Criollo-Mendoza et al., 2023). Alkaloids enhance the healing process by increasing collagen production and stimulating fibroblast activity, which is essential for tissue repair and regeneration. Additionally, alkaloids have been shown to reduce the number of inflammatory cells at the wound site, thereby mitigating excessive inflammation that can impede healing. This dual action of promoting collagen synthesis while concurrently limiting inflammation underscores the potential of alkaloids as effective agents in wound management, aligning with the broader therapeutic benefits associated with natural products rich in bioactive phytochemicals (Budiawan. et al., 2023).

This statistically significant result confirms that both the 75% Puting Anghel cream and the positive control group (aloe vera) had substantially better wound healing outcomes than the negative control, which did not receive an active treatment. When the 75% Puting Anghel group was compared to the positive control group, no significant difference was observed. This means that the wound healing performance of the Puting Anghel topical cream was comparable to that of the positive control, a commercially available and clinically recognized treatment (Aloe vera gel).

a.2. Acute Inflammation

In Table 10, an Analysis of Variance (ANOVA) was conducted to determine whether there were statistically significant differences in acute inflammation levels among the three groups: Treatment Group 1 (75% Puting Anghel topical cream), the positive control group (Aloe vera gel), and the negative control group (Cream Base only). Acute inflammation was assessed as a key criterion because it is a primary and early indicator of the body's healing response to injury; excessive or prolonged inflammation can hinder the wound healing process, while controlled inflammation typically promotes proper tissue repair. The test produced a p-value of 1.37×10^{-48} , which is far below the 0.05 significance level, indicating a highly significant difference in acute inflammation scores among the three treatment groups. This statistical result suggests that at least one group differed significantly in terms of inflammation response during wound healing.

As shown in Table 11, the comparison of acute inflammation scores across the treatment groups revealed notable findings. The 75% Puting Anghel topical cream and the positive control group (Aloe vera gel) both recorded a mean score of 1.0, indicating minimal acute inflammation characterized by scattered, small amounts of mixed inflammatory cells. In contrast, the negative control group presented a mean score of 2.0, suggesting moderate inflammation with denser cellular infiltration, particularly around blood vessels. These results demonstrate that the 75% Puting Anghel formulation was as effective as the positive control in reducing acute inflammation, as indicated by a p-value of 1.00, which is greater than the 0.05 significance level, showing no statistically significant difference between the two groups. Moreover, when either the 75% Puting Anghel group or the positive control group was compared to the negative control group,

the difference was statistically significant, with a p-value of 1.01×10^{-48} , which is less than the 0.05 significance level. This statistical outcome confirms that both the formulated topical cream and the commercially available product significantly reduced inflammation compared to the negative control group.

Wrightia antidysenterica (Puting Anghel) contains flavonoids, tannins, phenols, and alkaloids that contribute to its anti-inflammatory effect. According to Al-Khayri et al. (2022), flavonoids help reduce inflammation by limiting the release of inflammatory mediators. While tannins minimize inflammation by tightening tissues and reducing swelling (Moura de Melo et al., 2023). Phenols protect tissues by reducing oxidative stress and calming inflammatory responses (Puangpraphant et al., 2022). Alkaloids ease inflammation by regulating the body's immune responses (Bai et al., 2021). These combined actions support the effectiveness of the 75% Puting Anghel topical cream in reducing acute inflammation.

B. Epithelialization Time (Days)

In Table 12, the Treatment Group 1 has a mean epithelialization time of 12.7 days, the Positive Control Group has a mean epithelialization time of 12.3 days, and the Negative Control Group has a mean epithelialization time of 14 days. The positive control group had the least days until the scab fell off, and the negative control group had the most significant number of days. The control treated group which received the formulated Puting Anghel Topical Cream comes in between the positive control group and negative control group. Among the three treatments, the positive control group has the fastest wound healing response based on epithelialization time, followed by the Puting Anghel Topical Cream.

The p-value was 0.0723 (greater than 0.05), there was no statistically significant difference in epithelialization time among the three groups (75% concentration, positive control, and negative control), despite observable differences such as faster scab detachment in the 75% group. Thus, although the 75% topical cream and the positive control visually and histologically performed better than the negative control, the statistical test did not confirm a significant difference in epithelialization time.

Therefore, hypothesis no. 3 was rejected in terms of epithelialization time. The healing response, while possibly faster in appearance or tissue changes, was not statistically significant across the three groups.

Based on the histological evaluation and epithelialization time, the 75% Puting Anghel (*Wrightia antidysenterica*) Topical Cream demonstrated comparable effectiveness to Aloe vera gel. Both treatments showed equal scores in re-epithelialization, neovascularization, granulation tissue formation, fibroblast maturation within the granulation tissue, and acute inflammation, indicating that the Puting Anghel cream was equally effective as a standard wound burn treatment. Furthermore, when compared to the cream base alone, the 75% Puting Anghel formulation showed significantly greater effectiveness across all measured parameters, including a faster epithelialization time.

These findings suggest that the 75% Puting Anghel Topical Cream was particularly effective in promoting wound healing by enhancing tissue regeneration, reducing inflammation, and accelerating re-epithelialization.

Characteristics of the Prepared Topical Cream

Table 13 shows the evaluation of the prepared topical cream by its organoleptic properties (color, odor, and texture) and its pH.

A. Organoleptic Properties

The most commonly observed color of the topical cream prepared using the Puting Anghel plant was pale brown, as described by the three evaluators. This coloration was likely due to the presence of natural compounds such as flavonoids, tannins, or other phytochemicals inherent in the Puting Anghel extract. Pale brown was considered typical for plant-based creams, especially those derived from leaves or stems that undergo minimal processing. A consistent color across all observations suggests good formulation practices and proper blending of ingredients. According to Tan et al. (2022), the appearance of herbal creams, including their color, played a significant role in consumer perception and acceptance. Creams with uneven color may raise concerns regarding shelf life or active ingredient breakdown, which was not observed in this case. Since the color reflected the natural source of the plant, it aligned with expectations for a traditionally prepared topical remedy. Therefore, the pale brown color supported the product's identity as a natural, plant-based cream.

The odor of the cream formulated with Puting Anghel was described by 2 out of 3 evaluators as moderately aromatic. This type of scent was commonly associated with herbal preparations, indicating the presence of natural volatile oils and aromatic compounds from the plant. A moderately aromatic odor was favorable, as it was pleasant yet not overpowering for users. According to an article, by Perfumer & Flavorist, organoleptic characteristics, the natural odors of plant-based products were integral to their overall sensory profile and can influence consumer preferences. The cream's odor profile suggested that no artificial fragrances were added, allowing the natural aroma of Puting Anghel to be the dominant scent. The absence of any rancid or medicinal smell implies good ingredient quality and formulation methods. No negative responses were reported regarding the odor, supporting its acceptance. Thus, the odor of the cream was suitable and consistent with herbal product standards.

The topical cream made with Puting Anghel was consistently described by 2 out of three evaluators as smooth and creamy. This desirable texture indicated a successful emulsification of the cream's oil and water phases. Herbal extracts like Puting Anghel could sometimes introduce grittiness, but none was reported, indicating good filtration and formulation techniques. A smooth texture enhances the ease of application and supports the uniform distribution of active compounds on the skin. According to Navindgikar et al. (2020), texture is a critical parameter in topical formulations because it affects both user experience and the uniform distribution of active ingredients. Based on

the observations, the cream spread evenly and was easily absorbed, suggesting an optimal base and stabilizer ratio. This texture also helped ensure that the active compounds in Puting Anghel reached the skin surface efficiently. Therefore, the cream's texture was acceptable and supported its potential as a user-friendly topical product.

B. pH

The pH of the cream formulated with Puting Anghel extract was found to range between 4 and 6, based on pH strip testing. This mildly acidic range was ideal for topical products, as it closely matched the natural pH of human skin (around 5.5). Maintaining a skin-compatible pH helped protect the skin barrier, support microbial balance, and reduce the risk of irritation. According to Dreno et al. (2018), topical formulations should have preserved the acid mantle to prevent dryness and inflammation. A pH within this range was also beneficial for stabilizing plant-based active compounds, which might degrade in more alkaline conditions. The absence of reported irritation during evaluator testing supported that the cream was safe for skin contact. Jourdain et al. (2020) emphasized the importance of pH compatibility in herbal and cosmeceutical preparations. A stable pH also reflected proper formulation controls, including buffering and ingredient selection. Therefore, the measured pH confirmed that the cream was well-suited for topical use. It supported the potential of Puting Anghel as a safe, effective herbal treatment when formulated appropriately.

6. Conclusion

The findings of this study demonstrated the potential of Puting Anghel Topical Cream as a safe and effective treatment for wound healing. The Patch and Scratch test results confirmed that the cream had a Primary Irritation Index of zero (0), indicating no signs of irritation, erythema, or edema, thereby ensuring its safety for topical application. Histopathological examination and epithelialization time results revealed that the 75% concentration of Puting Anghel extract in the topical cream exhibited the most significant wound healing response, showing a faster epithelialization rate compared to the 50% and 25% concentrations. This suggested that the higher concentration of the extract was more effective in promoting quicker wound recovery. Statistical analysis showed no significant difference in histopathological outcomes between the 75% Puting Anghel cream and the positive control group (Aloe vera gel), suggesting comparable therapeutic effects. Both groups, however, exhibited significant improvements over the negative control (cream base only), supporting the efficacy of the active extract. While epithelialization time did not show statistically significant differences among the three groups, the 75% concentration still had the shortest healing duration based on mean values. Furthermore, the evaluation of the cream's organoleptic properties and pH level indicated that it was compatible with skin application. The cream's pale brown color, moderately aromatic odor, and smooth, creamy texture were found favorable by the evaluators, while the pH range of 4 to 6 aligned closely with the natural pH of the skin, supporting its suitability for topical use.

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9. Appendices

Appendix A

TIMETABLE

Parts	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Chapter 1: Introduction									
Chapter 2: Methodology									
Research and Design Methods									
Collection of Puting Anghel Leaves									
Extraction of Puting Anghel Leaves									
Rotary Evaporator Extraction									
Identification Testing Procedure for the Presence of Ethanol									
Phytochemical screening of Puting Anghel Leaf Extract									
Formulation of Puting Anghel Topical Cream									
Patch and Scratch Test									
Process of Burn Wound and Administration of Treatment									
Determination of the Wound Healing Response									
Chapter 3: Results and Discussion									
Chapter 4: Conclusions and Recommendations									

Appendix B

AUTHENTICATION CERTIFICATE OF PLANT MATERIAL



DON MARIANO MARCOS MEMORIAL STATE UNIVERSITY
North La Union Campus
Sapilang, Bacnotan 2515, La Union

COLLEGE OF AGROFORESTRY AND FORESTRY

IDENTIFICATION CERTIFICATE OF PLANT MATERIAL

This is to certify that Lynmarie Kate S. Abando, Menchie M. Damisil, Alnicko C. Obligacion, Jasmine Claire O. Castro, and Denise Josh R. Jamolo of the College of Pharmacy, Lorma Colleges, City of San Fernando, La Union have brought plant species for proper authentic identification. After a thorough and closer examination on the morphological and botanical characteristics of the specimen, it was identified and described as follows.

Common Name - Puting Anghel
Scientific Name - *Wrightia antidysenterica* (L.) R.Br.
Family Name - Apocynaceae

This certification is issued to Lynmarie Kate S. Abando, Menchie M. Damisil, Alnicko C. Obligacion, Jasmine Claire O. Castro, and Denise Josh R. Jamolo for all legal intentions and purposes.

Issued this 26th day of November 2024, College of Agroforestry and Forestry, Don Mariano Mariano Marcos Memorial State University, North La Union Campus, Bacnotan, La Union.

Prepared and examined by:


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

Noted:


FOR. JAY MARK G. CORTADO
Dean, CAFF




DR. JUNIFER REY E. TABAFUNDA
Chancellor

RELEASED
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DATE:
11/28/2024





Appendix C

BUREAU OF ANIMAL INDUSTRY (BAI) CERTIFICATION OF PET SHOP

	<p>Republic of the Philippines Department of Agriculture BUREAU OF ANIMAL INDUSTRY Visayas Avenue, Brgy. Vasra, Quezon City</p>				
<p>CERTIFICATE OF REGISTRATION</p>					
<p>also termed as</p>					
<p>LICENSE TO OPERATE</p>					
<p>consonance with the World Organisation for Animal Health (WOAH) and Organisation for Economic Cooperation and Development (OECD)</p>					
<p>Issued to</p>					
<p>PD RODENTS ANIMAL FARM</p>					
<p>Laboratory Animal Facility</p>					
<p>Barangay Bulaon Barrio, San Fernando City, Pampanga</p>					
<p>This facility is registered with the Bureau of Animal Industry pursuant to the provisions of the RA 3639 an Act creating the BAI, EO No. 292 Series of 1987 Administrative Code of 1987, EO No. 338 Series 2001 Agriculture and Fisheries Modernization Act, RA 8485 otherwise known as Animal Welfare Act of 1998, as amended by RA 10631 and RA 10611 Food Safety Act.</p>					
<table border="1"><tr><td>Date of Issuance</td></tr><tr><td>September 17, 2024</td></tr></table>	Date of Issuance	September 17, 2024	<table border="1"><tr><td>Valid Until</td></tr><tr><td>September 17, 2025</td></tr></table>	Valid Until	September 17, 2025
Date of Issuance					
September 17, 2024					
Valid Until					
September 17, 2025					
	<p>Signed by the Authority of the Director:</p> <p>ANTHONY C. BUCAD, DVM Veterinarian III Animal Health and Welfare Division</p>				
<p>AHWD-48 Animal Facility Registration Certificate Rev. No. 02 April 19, 2023</p>					

Appendix D

BUREAU OF ANIMAL (BAI) INDUSTRY PERMIT

 <p>Republic of the Philippines Department of Agriculture BUREAU OF ANIMAL INDUSTRY Visayas Avenue, Brgy. Vasra, Quezon City</p> <h3>ANIMAL RESEARCH CLEARANCE</h3>	
NAME OF INSTITUTION : LORMA COLLEGES	REFERENCE NO : <i>AR - 2024 - 0510</i>
	DATE/VENUE : January 2025 - May 2025 Lorma Colleges
BUSINESS ADDRESS : Carlatan, San Fernando City, La Union	LEAD RESEARCHER/VETERINARIAN/IACUC CHAIR: Menchie M. Damisil - Lead Researcher Conrado A. Apusen III, RPh – Veterinarian/IACUC Chair
<p>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 LORMA COLLEGES after completing the requirements to conduct the research entitled “Wound Burn Healing Property of Puting Anghel (<i>Wrightia antidysenterica</i>) Extract as Topical Cream in Wistar Albino Rats” on the date and venue stipulated above.</p> <p>The Institution is hereby reminded to observe the provisions of DA-AO no. 40 s.1999.</p> <p>Prepared on November 21, 2024</p>	
<p>Approved By Authority of the Director</p>  <p>HYACINTH G. NAPILOY, DVM, MPS-PA Chief, Animal Health and Welfare Division</p>	
<p>RF AHWD-49 Animal Research Clearance Rev. No. 03 April 19, 2023</p>	

Appendix E

CERTIFICATION OF EXEMPT FROM REC REVIEW



LC-REC Form #040
CERTIFICATE OF EXEMPTION FROM REVIEW

CERTIFICATION OF EXEMPTION FROM REVIEW


To: Lynmarie Kate S. Abando, Jasmine Claire O. Castro, Menchie M. Damisil, Denise Josh R. Jamolo and

Alnicko C. Obligacion

From: LORMA Colleges - Research Ethics Committee

Date: February 13, 2025

This is to certify that the Research Proposal entitled, "WOUND BURN HEALING PROPERTY OF PUTING ANGHEL (WRIGHTIA ANTIDYSENTERICA) LEAVES EXTRACT AS TOPICAL CREAM IN WISTAR ALBINO RATS" submitted by Lynmarie Kate S. Abando, Jasmine Claire O. Castro, Menchie M. Damisil, Denise Josh R. Jamolo and Alnicko C. Obligacion of College of Pharmacy has been reviewed by the Research Ethics Committee of LORMA Colleges and found that all ethical considerations are in place to conduct the research in the stated locale of the study. Hence, this research proposal is exempted from review.


RYAN JAY G. MISPOLES-MASE, RMT
Interim Chairman, LC REC/25

Appendix F

LIST OF MATERIALS, EQUIPMENT, AND REAGENT

MATERIALS

Puting Anghel leaves

Surgical tapes Shaver

Beaker

Round bottom flask

Stirring rod

Mortar and Pestle Dropper

Funnel

Water Bath

Soxhlet Apparatus

Rotary Evaporator

Oven

Weighing Balance

20-gauge hypodermic needle

Cylindrical steel rod (1 diameter)

Graduated Cylinder Thermometer

Test tube Test tube rack Filter paper

Chemicals

Ethanol

Spermaceti White wax Sodium borate

Sodium hydroxide solution

Lead acetate solution

1% gelatin solution

Sodium chloride

Ferric chloride solution

Diluted HCl

Mayer's reagent (potassium mercuric iodide)

Wagner's reagent (iodine in potassium iodide)

Dragendorff's reagent (potassium bismuth iodide)

Hager's reagent (saturated picric acid solution)

70% isopropyl alcohol

Chloroform

Dilute Sodium Hydroxide

0.05M Iodine

Appendix G

IODIFORM TEST

1. Test for the presence of ethanol

a. Iodoform Test

- Add a few drops of dilute sodium hydroxide, followed by adding 2 ml of 0.05 M iodine. The appearance of yellow precipitate indicates the presence of ethanol.

Appendix H

PHYTOCHEMICAL SCREENING PROCEDURES

1. Tests for the presence of Flavonoids

a. Alkaline Reagent Test

- Sodium hydroxide solution was added to the plant extract that resulting in a yellow fluorescence, which indicated the presence of flavonoids. The yellow color disappeared upon adding dilute sulfuric acid.

b. Lead Sulfide Test (Lead Acetate Test)

- A few drops of lead acetate solution were added to the plant extract. The appearance of a yellow precipitate confirmed the presence of flavonoids.

2. Test for the presence of tannins

a. Gelatin Test

- A 1% gelatin solution containing sodium chloride was added to the plant extract. The formation of a white precipitate confirmed the presence of tannins.

3. Tests for the presence of Phenols

a. Ferric Chloride Test

- The plant extract was treated with a few drops of ferric chloride solution. A bluish to black coloration indicated the presence of phenols.

4. Test for the presence of Alkaloids

The plant extract will be mixed with a small amount of diluted hydrochloric acid (HCl) and then filtered. The resulting filtrate subjected to the following tests:

a. Mayer's Test

- Mayer's reagent (potassium mercuric iodide) was added to the filtrate. The presence of alkaloids was confirmed by the formation of a yellow precipitate.

b. Wagner's Test

- The filtrate was treated with Wagner's reagent (iodine in potassium iodide). A reddish or brown precipitate indicated the presence of alkaloids.

c. Dragendorff's Test

- Dragendorff's reagent (potassium bismuth iodide) was added to the filtrate. The appearance of a red precipitate will signify the presence of alkaloids.

d. Hager's Test

- Hager's reagent (saturated picric acid solution) was added to the filtrate. The formation of a yellow precipitate indicated the presence of alkaloids.

Appendix I

PHYTOCHEMICAL ANALYSIS RESULTS

Test	Positive Result	Obtained Result	Interpretation
Flavanoids			
Alkaline Reagent Test	Formation of yellow fluorescence	There is a formation of yellow precipitate	+
Lead Sulfide Test (Lead Acetate Test)	Formation of yellow precipitate	There is a formation of yellow precipitate	+
Tannins			
Gelatin Test	Formation of white precipitate	There is a formation of white precipitate	+
Phenols			
Ferric Chloride Test	Formation of bluish to black coloration	There is a formation of black coloration	+
Alkaloids			
Mayer's Test	Formation of yellow precipitate	There is a formation of yellow precipitate	+
Wagner's Test	Formation of reddish or brown precipitate	There is a formation of brown precipitate	+
Dragendorff's Test	Formation of red precipitate	There is a formation of red precipitate	+
Hager's Test	Formation of yellow precipitate	There is a formation of yellow precipitate	+

Legend: (+) for Positive Result
(-) for Negative Result

Appendix J

FORMULATION OF CREAM BASE (USP 21- NF 16)

Formulation:

Spermaceti	6.25g
White wax	6g
Mineral Oil	28g
Sodium Borate	0.25g
Purified Water	9.5mL
To make	50 grams

Preparation:

1. Melt the spermaceti and white wax with mineral oil using a steam bath.
2. In another container, dissolve the sodium borate in water.
3. Add the sodium borate solution to the melted wax, stirring rapidly and continuously until a viscous white cream is formed.
4. Pour the cream into the container.
5. Allow it to congeal.
6. Cover the cold cream with a piece of circular glassine paper.

Appendix K

DOSAGE FORMULATION OF PUTING ANGHEL TOPICAL CREAM (USP 21- NF 16)

Formulation of 75% w/w concentration:

Putting Anghel Oil Extract 75 grams
Cream base 25 grams
To make 100 grams

Formulation of 50% w/w concentration:

Putting Anghel Oil Extract 50 grams
Cream base 50 grams
To make 100 grams

Formulation of 25% w/w concentration:







Putting Anghel Oil Extract 25 grams
Cream base 75 grams
To make 100 grams

Preparation:

1. Mix the ingredients, then triturate until desirable consistency is achieved.

Appendix L

PATCH AND SCRATCH TEST RESULT

Patch Test		
 <p>25%</p>	 <p>50%</p>	 <p>75%</p>
Scratch Test		
 <p>25%</p>	 <p>50%</p>	 <p>75%</p>

Appendix M

SCORING SYSTEM FOR HISTOPATHOLOGICAL EXAMINATION ON SKIN WOUND HEALING

Criteria	Score			
	0	1	2	3
Re-epithelialization	None	Partial	Complete, but immature or thin	Complete and mature
Neovascularization	None	Up to 5 vessels/HMF	6-10 vessels/HMF	>10 vessels/HMF
Granulation tissue amount	None	Scant (Involving <10 new vessel formations in 1 HMF)	Moderate (involving 11-20 vessel formation in 1 HMF)	Abundant (involving >20 new vessel formation in 1 HMF)
Granulation tissue fibroblast maturation	Immature	Mild maturation	Moderate maturation	Fully matured
Acute inflammation	None	Scant (scattered, small amount of mixed inflammation)	Moderate (moderate mixed inflammation concentrating around vessels)	Abundant (intensive mixed inflammation concentrating around vessels and forming clusters)
Note: HMF= High Power Magnification Fields				

Source: Sevimli-Gur, C., Erdogan, D., Onbasölar, I., Atilla, P., Çakar, N., & Gürhan, I. (2013). In vitro and in vivo investigations of the wound healing effect of crude Spirulina extract and C- phycocyanin.

Appendix N

SCORING FOR HISTOPATHOLOGICAL EXAMINATION ON SKIN WOUND HEALING

SCORING FOR HISTOPATHOLOGICAL EXAMINATION ON SKIN WOUND HEALING

	RE-EPITHELIALIZATION	NEOVASCULARIZATION	GRANULATION TISSUE AMOUNT	GRANULATION TISSUE FIBROBLAST MATURATION	ACUTE INFLAMMATION
1 CB	2	2	2	1	2
1 25%	2	2	2	2	2
1 50%	2	2	2	2	1
1 75%	3	2	2	3	1
1 AV	2	2	2	2	1
2 CB	1	2	2	1	2
2 25%	2	2	2	2	2
2 50%	2	2	2	2	1
2 75%	2	2	2	2	1
2 AV	3	2	2	3	1
3 CB	1	2	1	1	2
3 25%	2	2	2	2	2
3 50%	2	2	2	3	1
3 75%	3	2	2	3	1
3 AV	3	2	2	3	1

DAVE ~~FACTORS~~ ^{MD}, M.D., F.R.S.P.
 Pathologist
 LIC # 86729

Appendix O

HISTOPATHOLOGICAL EXAMINATION RESULTS

Groups	Results		
Group 1 - 75% Control Treated (Puting Anghel Topical Cream)	Control Treated 1 - 75% 	Control Treated 2 - 75% 	Control Treated 3 - 75%
Group 2 - 50% Control Treated (Puting Anghel Topical Cream)	Control Treated 1 - 50% 	Control Treated 2 - 50% 	Control Treated 3 - 50%
Group 3 - 25% Control Treated (Puting Anghel Topical Cream)	Control Treated 1 - 25% 	Control Treated 1 - 25% 	Control Treated 1 - 25%
Positive Control (Aloe Vera Gel)	Positive Control 1 	Positive Control 2 	Positive Control 3
Negative Control (Cream Base)	Negative Control 1 	Negative Control 2 	Negative Control 3

Appendix P

QUESTIONNAIRE TOOL FOR EVALUATION OF COLOR, ODOR & TEXTURE

Name (Optional): _____
 Course & Year/Position: _____

Date: _____

QUESTIONNAIRE TOOL FOR EVALUATION OF COLOR, ODOR & TEXTURE



Lorma Colleges
 Center for Health Sciences
 Carlatan, City of San Fernando, La Union
 College of Pharmacy



Evaluation Questionnaire for Formulated Topical Cream

Greetings with a LORMA Smile!

We are currently conducting a study titled **“Wound Burn Healing Property of Puting Anghel(*Wrightia antidysenterica*) Leaves Extract as a Topical Cream in Wistar Albino Rats”**, in partial fulfillment of the requirements in the subject PHARMACY RESEARCH METHODS WITH STATISTICS.

We kindly invite you to evaluate the formulated topical cream based on parameters such as color, odor and texture. Your insights and feedback are invaluable to our study. Your response will significantly contribute to the advancement of our research.

Thank you for your time and cooperation.

Best Regards,
The Researchers

Instructions for Evaluators : *Please complete the sections below based on your observations and experience. Evaluate the formulated topical cream according to the given parameters and mark your response in the corresponding boxes. You can also specify the answers if needed.*

Parameter	Question
Color	What is the color of the cream? <input type="checkbox"/> Vibrant White <input type="checkbox"/> Vibrant Green <input type="checkbox"/> Vibrant Brown <input type="checkbox"/> Vibrant Yellow <input type="checkbox"/> Pale White <input type="checkbox"/> Pale Green <input type="checkbox"/> Pale Brown <input type="checkbox"/> Pale Yellow

Odor	<p>How would you rate the intensity of the odor?</p> <input type="checkbox"/> Strong Aromatic Odor <input type="checkbox"/> Strong Herbal Odor <input type="checkbox"/> Moderate Aromatic Odor <input type="checkbox"/> Moderate Herbal Odor <input type="checkbox"/> Faint Aromatic Odor <input type="checkbox"/> Faint Herbal Odor
Texture	<p>1. How would you describe the texture?</p> <input type="checkbox"/> Smooth and creamy <input type="checkbox"/> Grainy <input type="checkbox"/> Oily <input type="checkbox"/> Thick <input type="checkbox"/> Light
<p>Additional Comments : <i>Please provide any further observations, recommendations, or feedback regarding the product's color, odor, or texture.</i></p>	
<div style="border: 1px solid black; height: 200px; width: 100%;"></div>	

Appendix Q



DOCUMENTATION OF SURVEY QUESTIONNAIRES WITH RESPONSES

Evaluator 1 - Faculty

Name (Optional): *Mr. Marc Benedict Panigo*
 Course & Year/Position: *COP Faculty*

Date: *04-01-2015*

QUESTIONNAIRE TOOL FOR EVALUATION OF COLOR, ODOR & TEXTURE

	Lorma Colleges Center for Health Sciences Carlatan, City of San Fernando, La Union College of Pharmacy	
Evaluation Questionnaire for Formulated Topical Cream		
<p>Greetings with a LORMA Smile!</p> <p>We are currently conducting a study titled "Wound Burn Healing Property of Puting Anghel (<i>Wrightia antidysenterica</i>) Leaves Extract as a Topical Cream in Wistar Albino Rats", in partial fulfillment of the requirements in the subject PHARMACY RESEARCH METHODS WITH STATISTICS.</p> <p>We kindly invite you to evaluate the formulated topical cream based on parameters such as color, odor and texture. Your insights and feedback are invaluable to our study. Your response will significantly contribute to the advancement of our research.</p> <p>Thank you for your time and cooperation.</p> <p>Best Regards, The Researchers</p>		
<p>Instructions for Evaluators : <i>Please complete the sections below based on your observations and experience. Evaluate the formulated topical cream according to the given parameters and mark your response in the corresponding boxes. You can also specify the answers if needed.</i></p>		
Parameter	Question	
Color	What is the color of the cream? <input type="checkbox"/> Vibrant White <input type="checkbox"/> Vibrant Green <input type="checkbox"/> Vibrant Brown <input type="checkbox"/> Vibrant Yellow <input type="checkbox"/> Pale White <input type="checkbox"/> Pale Green <input checked="" type="checkbox"/> Pale Brown <input type="checkbox"/> Pale Yellow	



Odor	<p>How would you rate the intensity of the odor?</p> <p><input type="checkbox"/> Strong Aromatic Odor</p> <p><input type="checkbox"/> Strong Herbal Odor</p> <p><input checked="" type="checkbox"/> Moderate Aromatic Odor</p> <p><input type="checkbox"/> Moderate Herbal Odor</p> <p><input type="checkbox"/> Faint Aromatic Odor</p> <p><input type="checkbox"/> Faint Herbal Odor</p>
Texture	<p>1. How would you describe the texture?</p> <p><input checked="" type="checkbox"/> Smooth and creamy</p> <p><input type="checkbox"/> Grainy</p> <p><input type="checkbox"/> Oily</p> <p><input type="checkbox"/> Thick</p> <p><input type="checkbox"/> Light</p>
<p>Additional Comments : <i>Please provide any further observations, recommendations, or feedback regarding the product's color, odor, or texture.</i></p>	
Empty space for additional comments	

Evaluator 2 - BS Pharmacy Second Year

Name (Optional): Stephanie Alba
Course & Year/Position: BS2-11

Date: 04-07-2025

QUESTIONNAIRE TOOL FOR EVALUATION OF COLOR, ODOR & TEXTURE

	<p>Forma Colleges Center for Health Sciences Carlatan, City of San Fernando, La Union College of Pharmacy</p>	
Evaluation Questionnaire for Formulated Topical Cream		
<p>Greetings with a LORMA Smile!</p> <p>We are currently conducting a study titled "Wound Burn Healing Property of Puting Anghel(<i>Wrightia antidysenterica</i>) Leaves Extract as a Topical Cream in Wistar Albino Rats", in partial fulfillment of the requirements in the subject PHARMACY RESEARCH METHODS WITH STATISTICS.</p> <p>We kindly invite you to evaluate the formulated topical cream based on parameters such as color, odor and texture. Your insights and feedback are invaluable to our study. Your response will significantly contribute to the advancement of our research.</p> <p>Thank you for your time and cooperation.</p> <p>Best Regards, The Researchers</p>		
<p>Instructions for Evaluators : <i>Please complete the sections below based on your observations and experience. Evaluate the formulated topical cream according to the given parameters and mark your response in the corresponding boxes. You can also specify the answers if needed.</i></p>		
Parameter	Question	
Color	What is the color of the cream? <input type="checkbox"/> Vibrant White <input type="checkbox"/> Vibrant Green <input checked="" type="checkbox"/> Vibrant Brown <input type="checkbox"/> Vibrant Yellow <input type="checkbox"/> Pale White <input type="checkbox"/> Pale Green <input type="checkbox"/> Pale Brown <input type="checkbox"/> Pale Yellow	



Odor	<p>How would you rate the intensity of the odor?</p> <input type="checkbox"/> Strong Aromatic Odor <input type="checkbox"/> Strong Herbal Odor <input checked="" type="checkbox"/> Moderate Aromatic Odor <input type="checkbox"/> Moderate Herbal Odor <input type="checkbox"/> Faint Aromatic Odor <input type="checkbox"/> Faint Herbal Odor
Texture	<p>1. How would you describe the texture?</p> <input type="checkbox"/> Smooth and creamy <input type="checkbox"/> Grainy <input checked="" type="checkbox"/> Oily <input type="checkbox"/> Thick <input type="checkbox"/> Light
<p>Additional Comments : <i>Please provide any further observations, recommendations, or feedback regarding the product's color, odor, or texture.</i></p>	
Empty space for additional comments	

Evaluator 3 - Other Department

Name (Optional): *Dmitri*
 Course & Year/Position: *BMCC 1-3*

Date: *09-07-2023*

QUESTIONNAIRE TOOL FOR EVALUATION OF COLOR, ODOR & TEXTURE

	<p>Lorma Colleges Center for Health Sciences Carlatan, City of San Fernando, La Union College of Pharmacy</p>	
<p>Evaluation Questionnaire for Formulated Topical Cream</p>		
<p>Greetings with a LORMA Smile!</p> <p>We are currently conducting a study titled “Wound Burn Healing Property of Puting Anghel(<i>Wrightia antidysenterica</i>) Leaves Extract as a Topical Cream in Wistar Albino Rats”, in partial fulfillment of the requirements in the subject PHARMACY RESEARCH METHODS WITH STATISTICS.</p> <p>We kindly invite you to evaluate the formulated topical cream based on parameters such as color, odor and texture. Your insights and feedback are invaluable to our study. Your response will significantly contribute to the advancement of our research.</p> <p>Thank you for your time and cooperation.</p> <p>Best Regards, The Researchers</p>		
<p>Instructions for Evaluators : <i>Please complete the sections below based on your observations and experience. Evaluate the formulated topical cream according to the given parameters and mark your response in the corresponding boxes. You can also specify the answers if needed.</i></p>		
<p>Parameter</p>	<p>Question</p>	
<p>Color</p>	<p>What is the color of the cream?</p> <ul style="list-style-type: none"> <input type="checkbox"/> Vibrant White <input type="checkbox"/> Vibrant Green <input type="checkbox"/> Vibrant Brown <input type="checkbox"/> Vibrant Yellow <input type="checkbox"/> Pale White <input type="checkbox"/> Pale Green <input checked="" type="checkbox"/> Pale Brown <input type="checkbox"/> Pale Yellow 	

Appendix R

CERTIFICATE OF STATISTICIAN



CERTIFICATION

This is to certify that the following Bachelor of Science in Pharmacy III students consulted the undersigned as **STATISTICIAN** of the Research entitled "***WOUND BURN HEALING PROPERTY OF PUTING ANGHEL (Wrightia antidysenterica) LEAVES EXTRACT AS TOPICAL CREAM IN WISTAR ALBINO RATS***".

ABANDO, LYNMARIE KATE S.

CASTRO, JASMINE CLAIRE O.

DAMISIL, MENCHIE M.

JAMOLO, DENISE JOSH R.

OBLIGACION, ALNICKO C.

Given this 23rd day of April, year Two Thousand and Twenty Five
at Lorma Colleges, Carlatan, City of San Fernando, La Union.

A handwritten signature in black ink, appearing to read "Joylyn P. Baniaga", is written over a horizontal line. The signature is stylized and cursive.

JOYLYN P. BANIAGA, LPT, MAME

Statistician

APPENDIX S

CERTIFICATE OF ENGLISH CRITIC

CERTIFICATION

This is to certify that the research entitled “**Wound Burn Healing Property of Puting Anghel (*Wrightia antidysenterica*) Leaves Extract as Topical Cream in Wistar Albino Rats**” by **Abando, Lynmarie Kate S., Castro, Jasmine Claire O., Damisil, Menchie M., Jamolo, Denise Josh R., and Obligacion, Alnicko C.**, third year students currently taking the degree of Bachelor of Science in Pharmacy of LORMA Colleges, had been checked for its grammatical structure and format by the undersigned.

This certification is issued to ensure that the institution received quality research work. Signed this 30th day of May 2025.



MICHELLE B. MACARIO, LPT, MA English
Master Teacher 1
Division Research Evaluator
Division of Abra

APPENDIX T

GRAMMAR AND PLAGIARISM CHECKER

Report: Thesis Group 6

Thesis Group 6

by WAG MAGDELETE NG HINDI SAYO OR ELSE VOID WARRANTY - ADMIN

General metrics

97,968	14,435	1032	57 min 44 sec	1 hr 51 min
characters	words	sentences	reading time	speaking time

Writing Issues

 No issues found

Plagiarism

 This text seems 100% original. Grammarly found no matching text on the Internet or in ProQuest's databases.

Unique Words

15%

Measures vocabulary diversity by calculating the percentage of words used only once in your document

unique words

Rare Words

52%

Measures depth of vocabulary by identifying words that are not among the 5,000 most common English words.

rare words

Word Length

5.5

Measures average word length

characters per word

Sentence Length

14

Measures average sentence length

words per sentence

APPENDIX U

ARTIFICIAL INTELLIGENCE AND PLAGIARISM CHECKER

8% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography
- Quoted Text
- Cited Text
- Small Matches (less than 12 words)
- Submitted works

Match Groups

- 56 Not Cited or Quoted 8%
Matches with neither in-text citation nor quotation marks
- 0 Missing Quotations 0%
Matches that are still very similar to source material
- 0 Missing Citation 0%
Matches that have quotation marks, but no in-text citation
- 0 Cited and Quoted 0%
Matches with in-text citation present, but no quotation marks

Top Sources

- 8% Internet sources
- 4% Publications
- 0% Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

No suspicious text manipulations found.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

*% detected as AI

AI detection includes the possibility of false positives. Although some text in this submission is likely AI generated, scores below the 20% threshold are not surfaced because they have a higher likelihood of false positives.

Caution: Review required.

It is essential to understand the limitations of AI detection before making decisions about a student's work. We encourage you to learn more about Turnitin's AI detection capabilities before using the tool.

Disclaimer

Our AI writing assessment is designed to help educators identify text that might be prepared by a generative AI tool. Our AI writing assessment may not always be accurate (it may misidentify writing that is likely AI generated as AI generated and AI paraphrased or likely AI generated and AI paraphrased writing as only AI generated) so it should not be used as the sole basis for adverse actions against a student. It takes further scrutiny and human judgment in conjunction with an organization's application of its specific academic policies to determine whether any academic misconduct has occurred.

Frequently Asked Questions

How should I interpret Turnitin's AI writing percentage and false positives?

The percentage shown in the AI writing report is the amount of qualifying text within the submission that Turnitin's AI writing detection model determines was either likely AI-generated text from a large-language model or likely AI-generated text that was likely revised using an AI-paraphrase tool or word spinner.

False positives (incorrectly flagging human-written text as AI-generated) are a possibility in AI models.

AI detection scores under 20%, which we do not surface in new reports, have a higher likelihood of false positives. To reduce the likelihood of misinterpretation, no score or highlights are attributed and are indicated with an asterisk in the report (*%).

The AI writing percentage should not be the sole basis to determine whether misconduct has occurred. The reviewer/instructor should use the percentage as a means to start a formative conversation with their student and/or use it to examine the submitted assignment in accordance with their school's policies.

What does 'qualifying text' mean?

Our model only processes qualifying text in the form of long-form writing. Long-form writing means individual sentences contained in paragraphs that make up a longer piece of written work, such as an essay, a dissertation, or an article, etc. Qualifying text that has been determined to be likely AI-generated will be highlighted in cyan in the submission, and likely AI-generated and then likely AI-paraphrased will be highlighted purple.

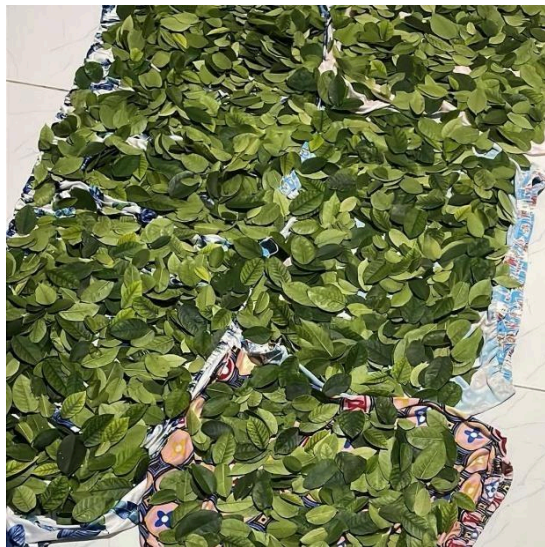
Non-qualifying text, such as bullet points, annotated bibliographies, etc., will not be processed and can create disparity between the submission highlights and the percentage shown.



APPENDIX V

PHOTO DOCUMENTATIONS OF PROCEDURES

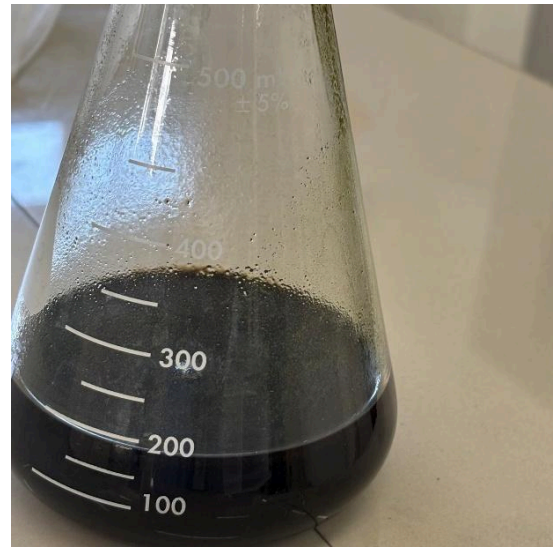
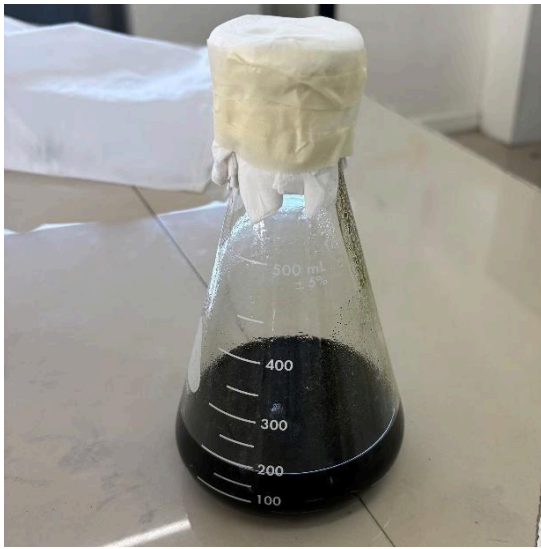
Collection of Puting Anghel Leaves



Preparation and Drying of Puting Anghel Leaves



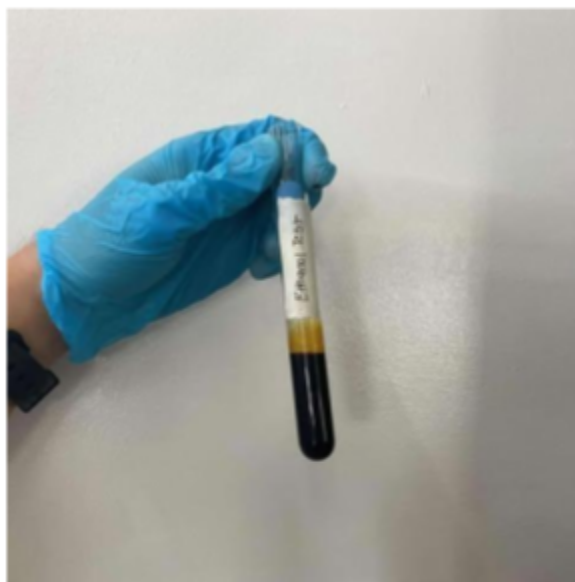
Extraction of Puting Anghel Leaves through Soxhlet Extraction



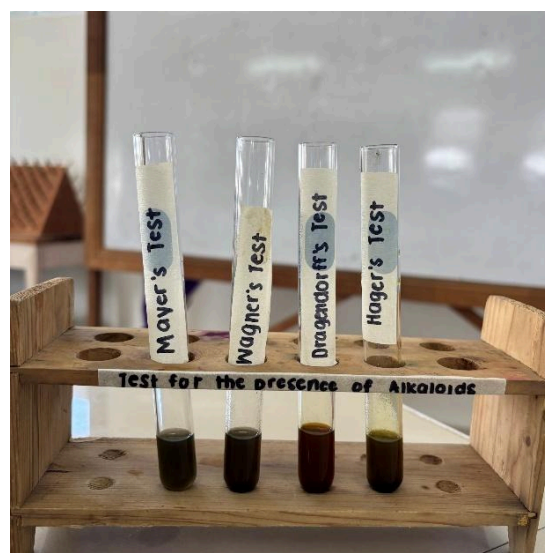
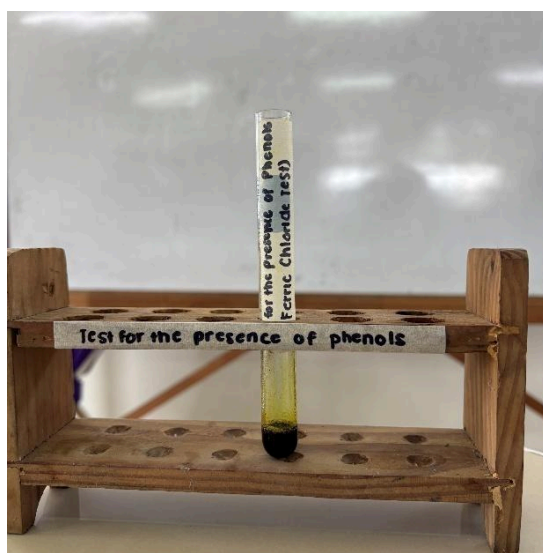
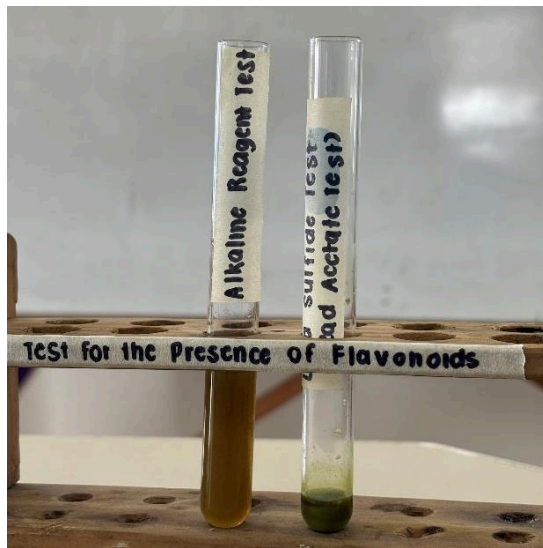
Removing Ethanol using Rotary Evaporator



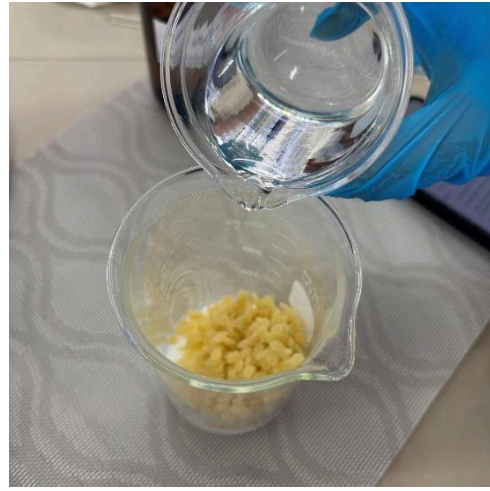
Ethanol Identification Test

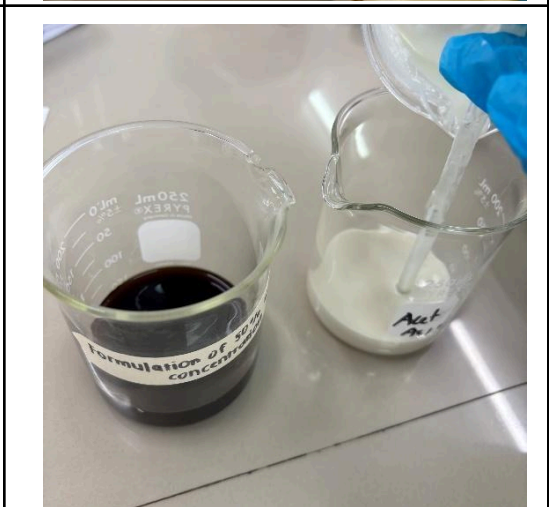
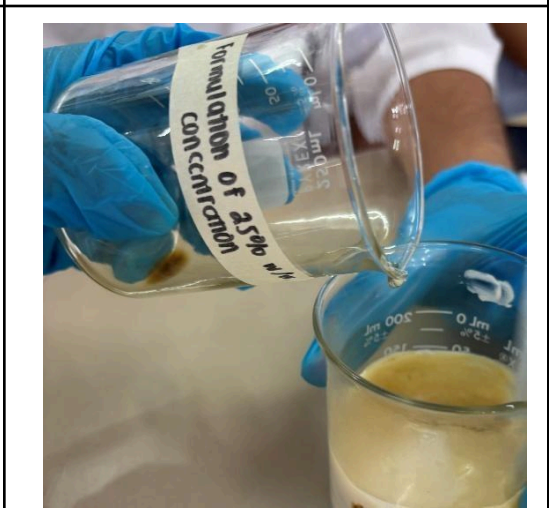


Phytochemical Analysis



Formulation of Creams







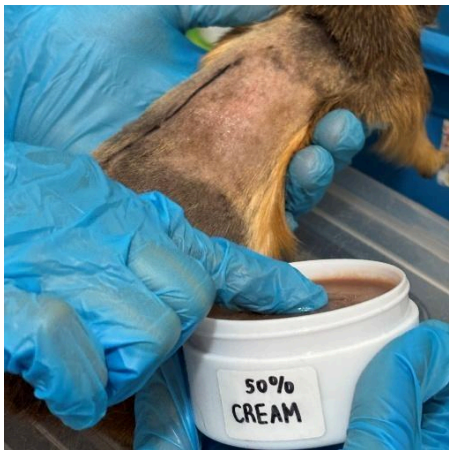
Patch and Scratch Test



Shaving and cleaning the skin of guinea pigs



Scratching the cleaned skin using 20-gauge hypodermic needle



Applying the assigned concentration (cream)



Covering the applied site with gauze



After 24hrs of applied Puting Anghel Cream
(From left to right 25%,50%,75%)

Infliction of Burn Wound



Anesthetizing the rats using Chloroform



Anesthetizing the rats using Chloroform



Shaving and cleaning the skin of the rats







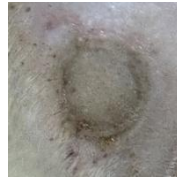

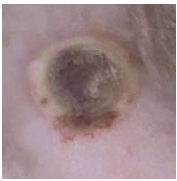




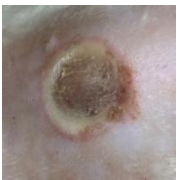


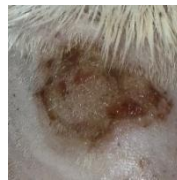

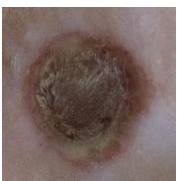
















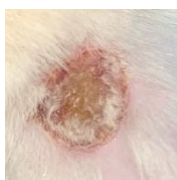






Measuring the boiling water to 100°C



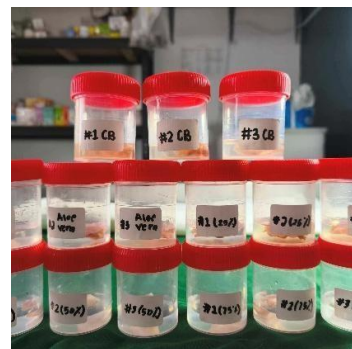
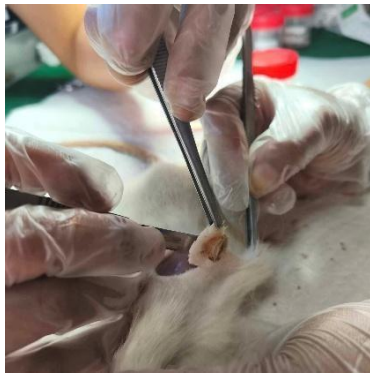
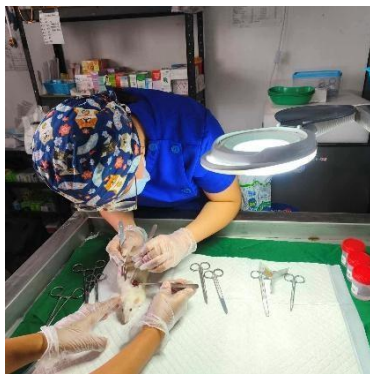
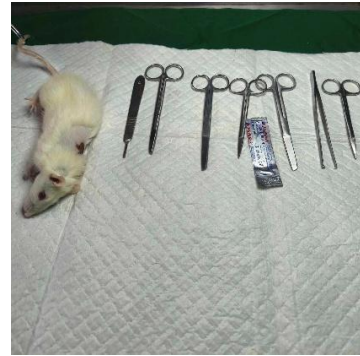
Inflicting burn wound to the cleaned skin using a 1cm cylindrical steel rod



Inflicted burn wound

Day	Treatment Group 75%	Treatment Group 50%	Treatment Group 25%	Positive Control Group	Negative Control Group
0					
1					
2					
3					
4					
5					
6					
7					

Collection of Skin Specimen for the Histological Examination



Questionnaires Survey



Faculty



Student, COP BSP 2



Student, Other Department

Photo-documentation on pH determination



The 75% Puting Anghel (*Wrightia antidysenterica*) Topical Cream demonstrated a pH range of 4 to 6.

10. Author(s)

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