



## Bioactivities of Purple Shamrock (*Oxalis Triangularis*) Crude Extract and Evaluation of Shamrock Topical Antibacterial Gel

Krizelle Joy B Dupra<sup>1</sup>, Robert S. Aceret MA<sup>2</sup>  
Saint Mary's University-Bayombong, Nueva Vizcaya, Philippines

**Corresponding Author:** Krizelle Joy B. Dupra, [krizellejoydupra@gmail.com](mailto:krizellejoydupra@gmail.com)

---

### ARTICLE INFO

*Keywords: Bacterial inhibition, non-irritant, skin microbiome, Topical Antibacterial Gel, Wound Healing*

*Received : 20, March*

*Revised : 25, April*

*Accepted: 29, May*

©2024 (s): Dupra, Aceret This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International](https://creativecommons.org/licenses/by/4.0/).



### ABSTRACT

This study delves into the intricate dynamics of the skin microbiome, focusing on its susceptibility to infection when the skin's protective barrier is compromised. Through a comprehensive examination of wound infections, the research underscores the prevalence of acute wounds and their profound impact on morbidity and mortality rates, particularly in developing nations like the Philippines. Despite adherence to WHO protocols, surgical site infections (SSI) remain alarmingly high, necessitating innovative interventions. Enter topical antibacterial agents, offering targeted therapy with minimal systemic impact. Leveraging the medicinal properties of *O. triangularis*, the study formulates and evaluates a topical antibacterial gel, unveiling its remarkable efficacy in wound healing and bacterial inhibition. Findings reveal promising results, exhibiting accelerated healing and notable antibacterial activity against *E. coli*. Moreover, its stability and non-irritant nature further accentuate its potential as a cost-effective solution for wound management. This research heralds a promising paradigm shift in wound care, advocating for the integration of botanical extracts in topical formulations for enhanced therapeutic outcomes.

---

## INTRODUCTION

The skin microbiome contains millions of bacteria, fungi, and viruses. The skin is the body's first line of protection against pathogens that might infect and harm humans. According to Belkaid *et al.* (2018), the phyla Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes comprise the majority of human skin microbiota. *Staphylococcus aureus* is the most common bacteria in the body's moist regions and belongs to the class Firmicutes (Carmona-Cruz *et al.*, 2022).

When the skin is burned, scratched, or surgically opened, microbes that dwell on the skin may take advantage of the opportunity to develop an infection. In the study of Bessa *et al.* (2015), twenty-eight (28) species were isolated from two hundred seventeen (217) infected wounds. The bacteria *Escherichia coli* and *S. aureus* were the most common germs detected in the wounds, respectively.

Wounds are classified into two types: chronic and acute. Acute wounds include cuts, burns, abrasions, and surgical wounds, which commonly heal by wound repair. Wound infections are responsible for 70-80% of fatalities following burn injuries and surgery (Abu Bakar *et al.*, 2019; Maskarinec *et al.*, 2019). An infected wound takes time to heal and impact one's quality of life. Wound infections are associated with patient mortality and morbidity, particularly in developing countries such as the Philippines.

In the study of Lipatan *et al.* (2020), they found out that there is a high surgical site infections (SSI) rate at 6.8% in mastectomy patients in the Philippine General Hospital. They found out that most patients who developed SSI were breast cancer patients. In the study of Arancel and Rovillos (2019), one hundred eighty-two (182) patients from various surgical wards in the Philippines were screened for the presence of healthcare-associated infections (HAI). It resulted in serious problems that have a negative impact on surgical results, expensive costs of medical care, and lengthening hospital stays. The duration of surgical operations is associated with a higher risk of HAI, particularly when SSI develop.

According to Barrasa *et al.* (2012), documented instances of SSI range from 8 to 30% in low-and middle-income countries (LMIC), where they are common but preventable complications. They found through the MEDIPINAS project, an international collaborative surgical initiative led by a group of Spanish surgeons, observed a high 28.8% rate of SSI despite World Health Organization (WHO) protocols in the Philippines.

Antibiotics that have been turned into a cream or ointment and are used topically are known as topical antibiotics. They are applied to shield mucosal membranes and skin from bacterial infections (Yalanis, 2022). Topical antibiotics are used to cure or prevent infections that may develop in minor cuts, abrasions, burns, impetigo, and surgical wounds (Bandyopadhyay, 2021). Topical treatments such as ointments or gels can effectively reduce pathogenic bacteria

on the skin and improve the regeneration of the skin to clear up scar formation (National Nutrition Council [NNC], 2021).

## THEORETICAL FRAMEWORK

Topical antibacterials represent a strategic approach to drug delivery, particularly advantageous for targeted therapy. By directly transporting the drug to the specific site of action, such formulations ensure optimal antibacterial bioavailability at the affected tissue, minimizing systemic exposure. This targeted delivery mechanism is particularly valuable in skin infections or localized bacterial overgrowths, allowing for more efficient and effective treatment. Additionally, the use of modest doses in topical antibacterial treatments contributes to reduced overall drug exposure, potentially minimizing the risk of systemic side effects. The cost-effectiveness of topical antibacterial agents further enhances their appeal, making them accessible and economically viable for various patient populations. Furthermore, since formulations are applied externally, they typically do not interfere with the normal microbial flora in the intestines, preserving the balance of the gut microbiome, which is crucial for overall health and immune function. This makes topical antibacterials a favorable option for localized infections, wound care, and dermatological conditions, combining precision in drug delivery with cost-effectiveness and minimal disruption to the body's microbial ecosystems. (Bandyopadhyay, 2021).

The research experiment started with the collection and preparation of the crude extract of *O. triangularis*. Purple shamrock was air dried, pulverized and macerated in 100ml of 80% ethanol. The plant extract was filtered using a paper filter and concentrated using a rotary evaporator at 45°C in 24 hours. The plant extract was subjected to phytochemical screening tests which includes flavonoids, steroids, phenols, tannins, alkaloids, cardenolides, anthraquinones, anthrones, coumarins, essential oils and sugars. The detected secondary metabolites had their R<sub>f</sub> values computed. The crude plant extract was also subjected into antibacterial testing against against *S. aureus* and *E. coli* using agar well method. The extract was quantified using activity index (AI) and relative percentage inhibition (RPI) and the MIC and MBC were determined (Guevara *et al.*, 2005). It was also subjected to egg albumin denaturation assay to test its anti-inflammatory activity (Madhuranga & Samarakoon, 2023).

Formulation of the topical antibacterial gel was based Rescober *et al.* (2022) using carboxymethylcellulose, gelatin and distilled water. Wound healing assay and dermal irritancy test was conducted on Sprague-Dawley rats to determine its wound contraction rate and non-irritant property (Don & Soosairaj, 2018; Rescober *et al.*, 2022). The researcher has substantial experience working with this animal specimen, having successfully conducted similar experiments in laboratory classes. This familiarity with rats ensures the ability of the researcher to effectively implement and interpret the proposed procedures in the context of

their research objectives. The post-surgical assessment was done using the Grimace Scale developed by Sotocinal *et al.* (2011). The evaluation of the topical antibacterial gel was composed of physical properties, pH level, test for thermal stability, spreadability, and phase separation.

This study mainly focused on the different bioactivities including anti-inflammatory, antibacterial and wound healing along with the formulation and evaluation of the topical antibacterial gel using the crude extract of purple shamrock. The formulation of the topical antibacterial gel only consists of carboxymethylcellulose (CMC), deionized water, and gelatin (Rescober *et al.*, 2022). The crude extract from the whole purple shamrock (roots, stem, leaves, and buds) will be using 80% ethanol for the extraction. It did not use any other solvent. The researcher only uses albino (Sprague-Dawley) rats when conducting the experiment.

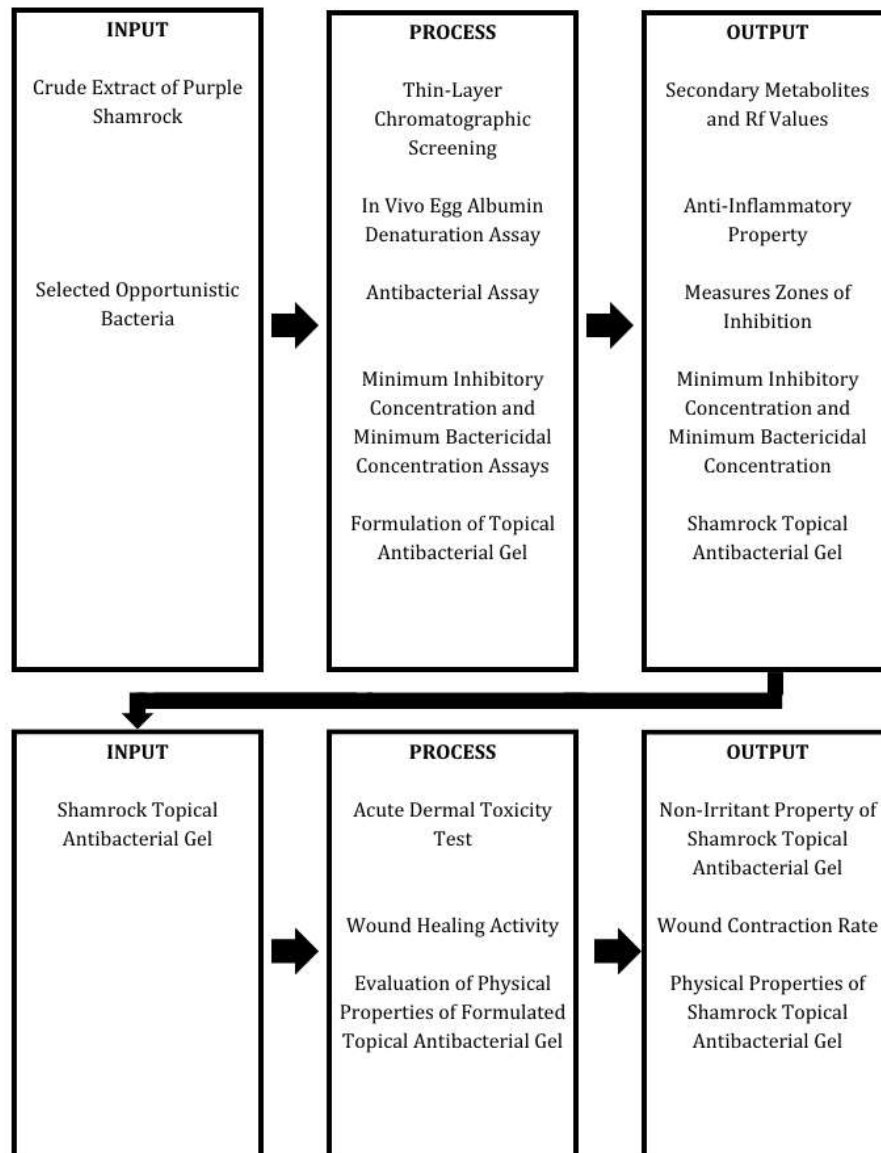


Figure 1. Conceptual Framework

## METHODS

### *Collection and Preparation of Purple Shamrock*

The plant samples were collected in the vicinity of San Roque, San Mateo, Isabelita wherein there is an abundance of Purple Shamrock. Each of the plant were carefully selected and examined to ensure that no pathogens are present. Purple shamrock was uprooted and left to dry.

### *Screening of Bioactive Compounds by Thin-Layer Chromatography*

The study utilizes the approach introduced by Guevarra *et al.* (2005). One hundred (100) grams of pulverized, dried purple shamrock was placed in an Erlenmeyer flask, and 80% ethyl alcohol was added to fully cover the plant material. The plant sample in the flask was covered with aluminum foil for 24-48 hours. The mixture when through filtration by gently pouring through a funnel with a Whatman filter paper and gauze pad. The plant sample was rinsed with fresh alcohol and was transferred to the funnel. Suction was moderately applied to complete the collection of *O. triangularis* crude extracts, and the plant waste was discarded. The filtrate was concentrated to 20 ml under vacuum at a temperature of 45°C (Guevarra *et al.*, 2005).

Thin-layer plates was prepared and 0.2 ml of ethanolic plant extracts was applied as spots, following the method described by Guevarra *et al.* (2005). Ethyl acetate-hexane with a ratio of 1:1 was used as a solvent system. Subsequently, the chromatogram was developed in silica gel G, and the resulting chromatogram was air-dried.

### *Determination of the R<sub>f</sub> Values*

To calculate R<sub>f</sub> values, measure the distance traveled by each separated compound from the baseline to the center of the spot or peak (spot center). Simultaneously, the total distance traveled by the solvent, from the baseline to the solvent front, must be measured. R<sub>f</sub> is then determined by dividing the distance traveled by the compound by the total distance traveled by the solvent. The resulting ratio is a dimensionless value that signifies the relative mobility of a compound in the given chromatographic conditions (PSIBERG, 2023).

### *In-Vitro Egg Albumin Denaturation Assay*

About 300 µg of diclofenac sodium powder was measured using a digital analytical balance and was added to 100.0 ml of distilled water, respectively. The solution was mixed well using a vortex. Two-fold dilution from 100 to 3.125 µg/ml was performed for *O. triangularis* crude extract and for the diclofenac sodium.

The positive control was made up of 0.2 ml of fresh egg albumin, 2.8 ml of buffered phosphate saline [PBS] (pH 7.4), and 2.0 ml of diclofenac sodium for a total volume of 5 ml. Diclofenac sodium is a NSAID that is used to treat mild-to-

moderate pain and helps to relieve symptoms of arthritis which usually are inflammation, swelling, stiffness, and joint pain (Alfaro & Davis, 2023).

Fresh egg albumin (0.2 ml), 2.8 ml of PBS (pH 7.4), and 2.0 ml of the crude extract of *O. triangularis* was mixed to make up the reaction mixture with a total volume of 5 ml.

The negative control was made up of 2.0 ml of distilled water, 0.2 ml of egg albumin and 2.8 ml of PBS (7.4 pH). Distilled water was used as a negative control because this shows no anti-inflammatory activity.

The three mixtures were then incubated at  $37 \pm 2^\circ\text{C}$  for 15 minutes and was be heated in a water bath at  $70 \pm 2^\circ\text{C}$  for 5 minutes. The reaction mixture was allowed to cool down at room temperature for 15 min. Absorbance of reaction mixture after denaturation was measured for each concentration (100  $\mu\text{g/ml}$ , 50  $\mu\text{g/ml}$ , 25  $\mu\text{g/ml}$ , 12.5  $\mu\text{g/ml}$ , 6.25  $\mu\text{g/ml}$  and 3.125  $\mu\text{g/ml}$ ) at 680 nm using a spectrophotometer. Each test was repeated thrice and the mean absorbance was recorded.

#### *Preparation and Standardization of Bacterial Broth Culture*

Nutrient broth was made by adding 13 g of nutrient broth powder (CM0001B) in 1L of distilled water. This must be mixed to dissolved the powder. Pour the mixture into a test tube and autoclave at  $121^\circ\text{C}$  for 15 minutes.

A loopful of *S. aureus* off the culture slant inoculate in a test tube filled with 50 ml of nutritional broth. The culture broth was incubated at  $35^\circ\text{C}$  for 18–24 hours. Turbidity of the culture broth was checked, as a sign of bacterial growth (Guevarra *et al.*, 2005). Five milliliters of the culture broth were transferred aseptically in sterile screw-capped tubes to regulate the turbidity of the inoculant. The bacterial suspension was stirred in a vortex mixer before comparing directly to the 0.5 McFarland standard. The bacterial broth may be adjusted by adding sterile nutrient broth. The new turbidity of the culture broth was compared to the McFarland standard until both have the same density (Guevarra *et al.*, 2005).

A loopful of *E. coli* off the culture slant inoculate in a flask filled with 50 ml of nutritional broth. The culture broth was incubated at  $35^\circ\text{C}$  for 18–24 hours. Turbidity of the culture broth was checked, as a sign of bacterial growth (Guevarra *et al.*, 2005). Five milliliters of the culture broth were transferred aseptically in sterile screw-capped tubes to regulate the turbidity of the inoculant. The bacterial suspension was stirred using a vortex mixer before comparing directly to the 0.5 McFarland standard. The bacterial broth may be adjusted by adding sterile saline solution or nutrient broth. The new turbidity of the culture broth was compared to the McFarland standard until both have the same density. (Guevarra *et al.*, 2005).

### *Antibacterial Assay*

Mueller-Hinton agar was prepared by suspending 38 g of Mueller-Hinton agar and 15 g of agar powder (bacteriological) in 1L of distilled water. This was brought to boil for the medium to be completely dissolved. The MH agar was transferred into a media bottle and was autoclaved at 121°C for 15 minutes.

Five plate replicates were prepared for each test organism. Twenty milliliter of melted Mueller-Hinton agar was poured into dry and sterile petri dishes. After drying, the sterile cotton swab was moistened into the inoculum suspension. It was firmly pressed and rotated to remove any excess liquid. The cotton swab was then inoculated in the dried MH agar.

For the position of the agar wells in the plate, trace an outline of a circular petri dish onto a piece of white paper. Draw 6 small circles in 6 mm diameter separated by an angle of 60° around the periphery of the circle (Guevarra *et al.*, 2005). After 20 minutes, wells with 6 mm diameter will be made with the help of a sterile cork borer into the plates and was filled with the plant extract (50 µg/ml), the positive control and the negative control.

The positive and negative control wells will be filled with clindamycin and ciprofloxacin for *S. aureus* and *E. coli* (standard drug at 50 µg/ml) and 80% ethyl alcohol (50 µg/ml) respectively. The plates were incubated for 24 hours under 37°C. This was done in five replicates in both test organisms.

### *Determination of the Minimal Inhibitory Concentration*

Mueller-Hinton broth was prepared by suspending 21g of Mueller Hinton broth powder (CM0405B) in 1L of distilled water. This was mixed to completely dissolved the powder into the water. It was poured into a media bottle and was sterilized by autoclaving at 121°C for 15 minutes.

Several colonies of *S. aureus* were transferred to a tube with 5ml of MH broth. This preculture was incubated for 24 hrs. at 37°C in an incubator. For the preparation of test inoculum, 0.15 ml of the preculture was transferred to 10 ml of MH broth, (Luber *et al.*, 2003).

In a separate tube, several colonies of *E. coli* were transferred to a tube with 5ml of MH broth. This preculture was incubated for 24 hrs. at 37°C in an incubator. For the preparation of test inoculum, 150 µl of the preculture was transferred to 10 ml of MH broth (Luber *et al.*, 2003).

The 96-well microtitration plate were all filled with 50 µg/ml of uncultured MH broth. The first row of the microtitration plate was filled with broth only. The second row was filled with the negative control, 80% ethyl alcohol. The 3<sup>rd</sup> row was filled with the positive control, clindamycin for *S. aureus*

and ciprofloxacin for *E. coli*. Fifty micrograms over milliliter ( $\mu\text{g}/\text{ml}$ ) of purple shamrock crude extract was suspended in the 4<sup>th</sup> to last rows of the 96-well microtitration plate. It undergone a two-fold dilution process (100, 50, 25, 12.5...  $\mu\text{g}/\text{ml}$ ) until it reaches the 12<sup>th</sup> column (European Committee for Antimicrobial Susceptibility Testing [EUCAST], 2003; Zarai *et al.*, 2011).

The first row of the 96-well microtitration plate was filled with broth only. This is to showcase that all of the wells have growth in them. The third row was filled with clindamycin for *S. aureus* and ciprofloxacin for *E. coli*. Fifty microgram per millimeter was dispensed in the wells. The positive controls must not show bacterial growth of *S. aureus* and *E. coli* (EUCAST, 2003; Zarai *et al.*, 2011). The second row of the 96-well microtitration plate was the negative control, 80% ethyl alcohol. Fifty micrograms per millimeter was dispensed in the wells.

The 96-well microtitration plate was all filled with 10  $\mu\text{g}/\text{ml}$  of *S. aureus* and *E. coli* bacterial suspension (EUCAST, 2003; Zarai *et al.*, 2011). The plates were covered with sterile plate and will be incubated at 37°C for 24 hours.

After that, 40  $\mu\text{l}$  of MTT at a final concentration of 5 mg/ml was added to each well and the mixtures were incubated at 37°C in 30 minutes. The change of color into purple indicated that the bacteria are biologically active.

#### *Determination of Minimal Bactericidal Concentration*

The wells with no turbidity were selected and was inoculated onto the surface of MHA plates. The plates were incubated for 24h at 37°C, wherein MBC was taken as the lowest concentration of the substance at which no colonies formed under these conditions.

#### *Preparation of Topical Antibacterial Gel*

The hydrogel-based gel was adapted from Rescober *et al.* (2022). Ten grams of carboxymethylcellulose (CMC) was gradually dissolved in 20 ml distilled water and 10 g of gelatin was added in 20 ml hot distilled water. Once dissolved, the gelatin solution was gradually added to the CMC solution and was continuously stirred until it becomes homogenous. The 5 ml of *O. triangularis* crude extract was gradually added to the CMC-gelatin solution with continuous stirring. A sufficient volume of distilled water was added to the mixture to achieve the desired 100 ml volume.

#### *Acute Dermal Toxicity Test*

Fur was removed about a day prior to the test by using a suitable depilatory material to carefully clip the dorsal portion of the trunk on both flanks. To avoid abrading the skin, extra care was used. A day before to the toxicity test procedure, the skin was cleansed with sterile water.

To ensure good contact and uniform distribution of the test chemical on the skin for the duration of the exposure period (24 hours), .5 ml of the topical antibacterial gel was applied once on the rat using a 1 ml syringe (Rescober *et al.*,

2022). Initial testing was performed on a single rat, and a confirmatory test was subsequently carried out on two more rats at the same time after monitoring the first rat's response for 24 hours. At the end of the exposure period, the gel was removed, and the rats were observed for 7 days for development of any adverse skin reactions like inflammation, irritation, or redness (Tazeze *et al.*, 2021). Testing site was examined using Draize criteria for symptoms of erythema and edema for 24 hrs., 48 hrs., and 72 hrs.

Each rat was monitored throughout the entire procedure, from the point when the procedure could impact the welfare of the animal, until complete recovery, and monitoring was documented. Monitoring included scoring sheets that have been developed based on validated assessment to measure and assess a rat's condition. The Rat Grimace Scale, a system of coding facial expressions as a measure of spontaneous pain, was utilized by the researcher to quantify some post-procedural pain.

#### *Wound Healing Activity*

Since most rats used in clinical trials are inbred, their genetic makeup is nearly same, which contributes to more consistent outcomes. The fact that their biological, genetic, and behavioral traits closely mimic those of humans is another factor contributing to their employment as models in medical testing. For the wound healing activity, rats were used to observe the wound contraction rate at a larger scale and give more accurate reading compared to mice. The rat was used in observing the acute wound healing activity of the formulated topical antibacterial gel because it is relatively cost-efficient, easy to handle and maintain and are bigger than mice (Grada *et al.*, 2018). This allowed the researcher to have more area for dermal application and experimentation. Rats also heal predominantly by wound contraction.

Three random rats weighing 150 g were utilized in the wound healing activity (Rescober *et al.*, 2022). An initial testing was performed on a single rat (Rat B1), and a confirmatory test was subsequently carried out on two more rats (Rat B2 and B3) at the same time after monitoring the first rat's response for 24 hours.

A rat weighing 150 g was acclimatized minimum to 3 days prior to use in surgery or long-term experiments. The acclimatized rat was anesthetized with 5% lidocaine, with its back hairs shaved using a razor blade, and the surgical site was disinfected with normal saline solution [NSS] (Rescober *et al.*, 2022).

The rat was placed in a lateral position followed by inducing two punch biopsies of 4-mm diameter area of wound on the shaved skin using a disposable punch biopsy tool (Rescober *et al.*, 2022). The distances between one punch biopsy to another were between 10 mm. The wound was cleaned with cotton swabs soaked in normal saline solution [NSS] (Rescober *et al.*, 2022). One milliliter of the topical antibacterial gel, hydrogel base, and povidone-iodine were applied

using a 1cc syringe without the needle every 24 hours. The wound was sealed with gauze and a micropore to cover (Dons & Soosairaj, 2018).

Each rat was monitored throughout the entire procedure, from the point when the procedure impacted the welfare of the animal, until complete recovery, and monitoring was documented. Monitoring included scoring sheets that have been developed based on validated assessment to measure and assess a rat's condition. The Rat Grimace Scale, a system of coding facial expressions as a measure of spontaneous pain, was utilized by the researcher to quantify some post-procedural pain. The rats were also be weighted every day for observable changes.

The wound was also measured during the 2<sup>nd</sup>, 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup>, and 18<sup>th</sup> day using a Vernier caliper. Wound contraction rate (WCR) is used in wound healing to assess the progress of healing, reduce wound size, minimize scar tissue formation, lower infection risk, improve cosmetic outcomes, guide treatment decisions, and predict healing time, all of which are critical for effective wound management and optimal recovery.

#### *Evaluation of the Physical Characteristics of the Formulated Topical Antibacterial Gel*

##### *1. Determination of physical properties*

The gel was observed for color, odor, and appearance (Arakareddy *et al.*, 2020).

##### *2. pH of the gel*

The pH level of the antibacterial gel was determined by using digital pH meter. About 1 g of the gel was weighed and dissolved in 100 ml of distilled water and stored for two hours. The measurement of pH of each formulation were done in five replicates and average value was calculated (Arakareddy *et al.*, 2020).

##### *3. Test for thermal stability*

The formulated topical antibacterial gel was inserted into glass bottles with the help of spatula and taped to settle to the bottom. Fill up to two-third capacity of bottle and insert plug and tighten the cap. Filled bottle was kept erect inside the incubator at 4 °±1 ° for 48 h. The sample passed the test, if on removal from the incubator shows no oil separation or any other phase separation (Arakareddy *et al.*, 2020).

##### *4. Spreadability*

This method was taken from Rescober *et al.* (2022). The formulated antibacterial gel was placed in between two glass slides and slipped off in which the spreadability (s) was calculated using the formula:

$$S = \frac{M + L}{T}$$

Wherein:

M= weight of substance placed on top of the glass slide

L= the length travelled

T= the time taken to separate two glass slides

#### 5. Phase separation

This method was taken from Arakareddy *et al.* (2020) with some modifications. The formulated topical antibacterial gel was kept intact in a closed container at 25–30 °C not exposed to light. Phase separation was observed carefully every 24 h for 15 d. Any change in phase separation was checked.

#### Treatment of Data

The ratio of the spot's travel distance between its application point and its center to the solvent's travel distance, multiplied by 100, is the spot's Rf (PSIBERG, 2022). Mathematically stated:

$$Rf = \frac{\text{Distance of solute from points of application}}{\text{Distance of solvent from points of application}} \times 100$$

For the anti-inflammatory assay, the Microsoft Excel was used to calculate the mean and the standard error of the mean of the three replicates. Nonlinear regression was applied using the Microsoft Excel to determine the formula and R<sup>2</sup> to calculate the half-maximal inhibitory concentration (IC<sub>50</sub>) value and concentration relationships.

The percentage inhibition of protein denaturation was be calculated using the formula;

$$(\%) \text{ Percentage Inhibition} = (A_c - A_t / A_c) \times 100$$

Where:

A<sub>t</sub>: the absorbance of the test.

A<sub>c</sub>: is the absorbance of the negative control.

All the data was expressed as a mean ± standard deviation of the five replicates of petri dishes containing culture media and test microorganisms. The antibacterial assay was assessed by measuring the diameter of the zone of inhibition (in mm). Activity index (AI) was calculated using the formula:

$$\text{Activity index (AI)} = \frac{\text{Inhibition Zone of the sample}}{\text{Inhibition of the standard}}$$

Determination of the relative percentage inhibition (RPI) with respect to the positive control was calculated using the following formula;

$$RPI = \frac{100(X - Y)}{(Z - Y)}$$

Where:

X=Total area of inhibition of the test extract

Y: Total area of the inhibition of the solvent

Z: Total area of the inhibition of the standard drug

The total area of the inhibition was calculated using area:  $A = \pi r^2$ ; where, r=radius of the zone of inhibition.




The Draize criteria used by Ankomah *et al.* (2022) was used to evaluate for observable signs of erythema and edema.

Table 1.  
Draize Criteria

Score	Translation
0	No erythema or edema
1	Very inappreciable edema or erythema
2	Small edema with raised skin at the edges of the area
3	Moderate to severe erythema or edema
4	Severe erythema or edema

The rat grimace scale, developed by Sotocinal *et al.* (2011), was utilized to quantify the post-procedural pain. Below is the scoring sheet provided by the National Centre for the Replacement, Refinement and Reduction of Animals in Research.

Table 2.  
Grimace Scale

	Not Present "0"	Moderately Present "1"	Obviously Present "2"
<b>Orbital Tightening</b> <ul style="list-style-type: none"> <li>• Closing of eyelid (narrowing of orbital area)</li> <li>• A wrinkle may be visible around the eye</li> </ul>			

### Nose/Cheek Flattening

- Flattening and elongation of the bridge of the nose
- Flattening of the cheeks (potentially sunken look)



### Ear Changes

- Ears curl towards and are angled forward to form a "pointed" shape
- Space between the ears increases



### Whisker Changes

- Whiskers stiffen and angle along the face
- Whiskers may clump together
- Whiskers lose their natural downward curve



The wound reduction area was monitored on predetermined days (2<sup>nd</sup>, 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup>, and 18<sup>th</sup> day) and measured using a Vernier caliper (Rescober *et al.*, 2022).

WCR was calculated using the formula provided wherein it is the percentage of the reduction in original wound area size as measured using a Vernier caliper in millimeters (Dons & Soosairaj, 2018; Rescober *et al.*, 2022).

$$WCR = \left( \frac{\text{Initial wound area} - \text{Nth day wound area}}{\text{Initial wound area}} \right) (100)$$

## RESULTS

Table 3.

*Detected Secondary Metabolites and their Rf Values from Powdered O. triangularis*

Compounds Tested	Reagent	Result	Rf Values
Essential Oil	Preliminary Test	+	.44
Triterpene and Sterols	Vanillin sulfuric acid	-	-
Phenols	Vanillin sulfuric acid	+	.38
Fatty Acids	Vanillin sulfuric acid	+	.83
Sugars	@-Nahthol-sulfuric acid	-	-
Anthraquinones	Methanolic potassium hydroxide (KOH-MetOH)	-	-
Coumarins	Methanolic potassium hydroxide (KOH-MetOH)	+	.49
Anthrones	Methanolic potassium hydroxide (KOH-MetOH)	-	-
Tannins	Potassium ferricyanide-ferric chloride	-	-
Flavonoids	Potassium ferricyanide-ferric chloride	-	-
Alkaloids	Dragendorff's reagent	-	-
Glycosidic Flavonoids	Antimony (III) chloride	+	.97
Steroids	Antimony (III) chloride	+	.88

(+) present, (-) absent

Table 3 shows the detected secondary metabolites and their Rf values from the powdered *O. triangularis*. From the different compounds tested, only 6 compounds were positive which were essential oils, phenols, fatty acids, coumarins, glycosidic flavonoids, and steroids. The detection of these six classes of secondary metabolites in the powdered *O. triangularis* indicates the extraction of a diverse array of bioactive compounds, each with its potential health benefits and pharmacological properties.

Figure 2.  
 Percentage Inhibition of Egg Albumin Denaturation of *O. triangularis* Crude Extract and Standard Drug (Diclofenac Sodium)

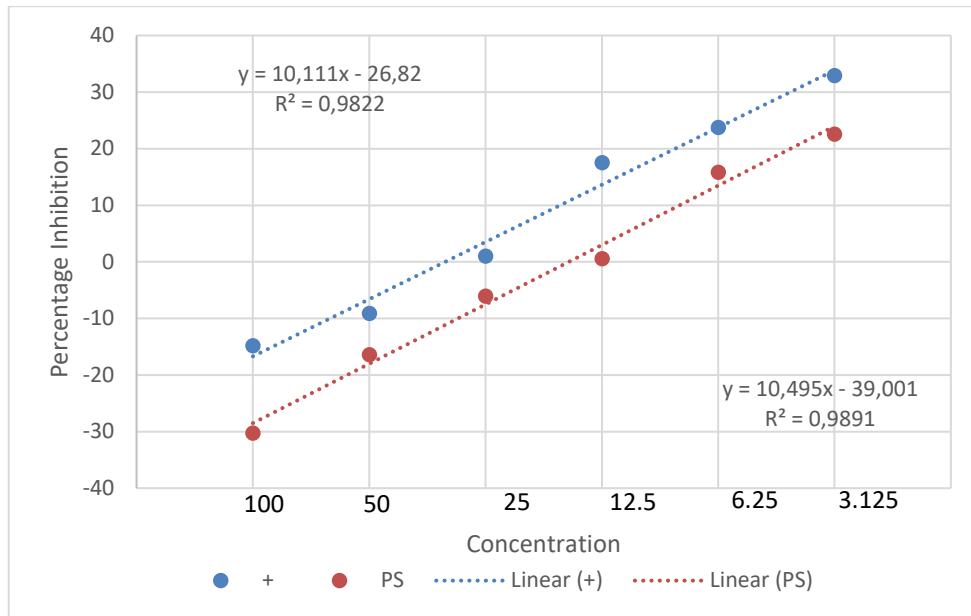


Figure 2 shows the percentage inhibition of *O. triangularis* crude extract and the standard drug, diclofenac sodium under a linear regression x-y scatter plot. The standard drug, diclofenac sodium, exhibited potent inhibition of protein denaturation than purple shamrock crude extract.

Table 4  
 IC50 Values of *O. triangularis* Crude Extract and Standard Drug (Diclofenac Sodium)

Treatment	Coefficient of Determination (R <sup>2</sup> )	IC <sub>50</sub> Values
Diclofenac Sodium	.9822	7.6
<i>O. triangularis</i> Crude Extract	.9891	8.48

Table 6 shows the half-maximal inhibitory concentration (IC<sub>50</sub>) of the crude extract and the standard drug. This serves as a crucial and commonly employed metric for gauging a drug's effectiveness. It signifies the quantity of a drug required to impede a biological process by 50%, thereby offering insight into the potency of an antagonist drug in pharmacological investigations (Aykul & Martinez-Hackert, (2016). Diclofenac sodium has a low IC<sub>50</sub> value than the *O. triangularis* crude extract signifying that it can effectively inhibit the biological process at low concentrations (Berrouet et al., 2020). Even though the *O. triangularis* crude extract has a higher IC<sub>50</sub> value than the standard drug, it still fights inflammation effectively since its IC<sub>50</sub> value is close to the standard drug.

Table 5

Minimum Inhibitory Concentration of *O. triangularis* Crude Extract Against the Test Organisms for 24 h

Test Organism	Concentration ( $\mu\text{g/ml}$ )							
	30	25	20	15	10	5	3	1
<i>S. aureus</i>	+	+	+	+	-	-	-	-
<i>E. coli</i>	+	+	+	-	-	-	-	+

Positive (+): Turbidity indicating growth; Negative (-): No turbidity indicating absence of growth

In this study, the MIC of *O. triangularis* against *E. coli* were determined by microdilution method and was found to be effective at 15-3  $\mu\text{g/ml}$ . The MIC of *O. triangularis* against *S. aureus* were determined by microdilution method and found to be effective at 10-1  $\mu\text{g/ml}$ . This is the first study in the literature to include the MIC of the crude extract of *O. triangularis* against *E. coli* and *S. aureus*.

Table 6

Minimum Bactericidal Concentration of *O. triangularis* Crude Extract Against the Test Organisms for 24 h

Test Organism	Concentration ( $\mu\text{g/ml}$ )							
	30	25	20	15	10	5	3	1
<i>S. aureus</i>	+	+	+	+	+	+	+	+
<i>E. coli</i>	+	+	+	+	+	-	-	+

Positive (+): Turbidity indicating growth; Negative (-): No turbidity indicating absence of growth

The MBC of the crude extract of *O. triangularis* were determined by inoculating the wells with no turbidity on MHA plates and incubated for 24 hours. Only 5-3  $\mu\text{g/ml}$  of the crude extract against *E. coli* were found to completely inhibit and eradicate its growth. This simply implies that *O. triangularis* exhibits bactericidal activity against *E. coli*.

Table 7

Rat Grimace Scale on Acute Dermal Toxicity Assay

Criteria	Rat-A1	Rat-A2	Rat-A3
Orbital Tightening	0	0	0
Nose/Cheek Flattening	0	0	0
Ear Changes	0	0	0
Whisker Changes	0	0	0

Table 12 shows the rats did not exhibit any signs of pain when the formulated topical antibacterial gel was applied on their back. There were no signs of orbital tightening, nose/cheek flattening, ear changes, and whisker changes. All rats exhibited a normal behavior after the assay.

Table 8  
*Initial Test-Draize Criteria*

Rats	(March 16) 24 hrs		(March 17) 48 hrs		(March 18) 72 hrs	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
Female (A1)	0	0	0	0	0	0

Table 9 shows that rat A1 did not exhibit any erythema or edema for the past three days.

Table 9  
*Confirmatory Test-Draize Criteria*

Rats	(March 17) 24 hrs		(March 18) 48 hrs		(March 19) 72 hrs	
	Erythema	Edema	Erythema	Edema	Erythema	Edema
Female (A2)	0	0	0	0	0	0
Female (A3)	0	0	0	0	0	0

Table 9 shows that rat A1 and rat A2 did not exhibit any erythema or edema for the past three days. The confirmatory test was started after Rat A1 was observed for 24 hours. Two albino rats (Rat A2 and Rat A3) served as the confirmatory test. The formulated topical antibacterial gel was removed using cotton balls soaked with NSS.

Figure 3  
*Wound Contraction Rate of Rats from Day 0 to Day 16*

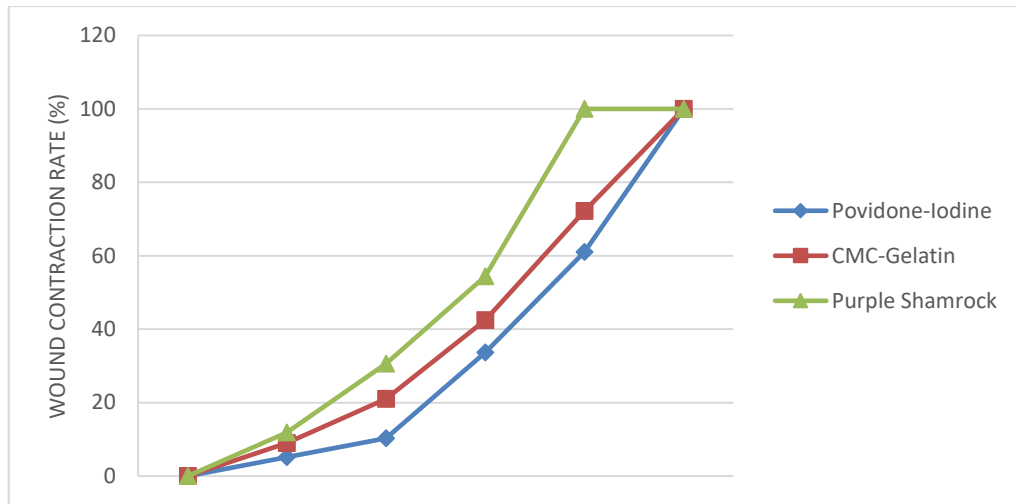





Figure 3 shows the contraction rate of the wounds between day 0 to day 16. The shamrock topical antibacterial gel exhibited the fastest wound healing activity. Arakelyan and Arakelyan (2020) reported that *O. triangularis* have benefits when turned into an ointment for cuts, scrapes, rashes and skin infections. Additionally, Mohammadi-Cheraghbabadi and Hazrati (2023) also reported that secondary metabolites such as phenolics, fatty acids, as well as glycosides can facilitate the wound-healing process by exerting pharmacological effects on the body. This signify that *O. triangularis* crude extract can heal wounds.

Rescober *et al.* (2022) reported that CMC-Gelatin solution did exhibit some wound healing property but not as significant as the plant extract. The rat treated with povidone-iodine, exhibited the slowest wound contraction. Povidone iodine is an effective antiseptic that does not impede wound healing (Bigliardi *et al.*, 2017). Additionally, iodine is an effective antiseptic agent that shows neither the purported harmful effects nor a delay of the wound-healing process, particularly in chronic and burn wounds (Vermeulen *et al.*, 2010).

Table 10  
Wound Contraction Rate of the Rats from Day 0 to Day 16

Day	Rat B1	Rat B2	Rat B3
0			

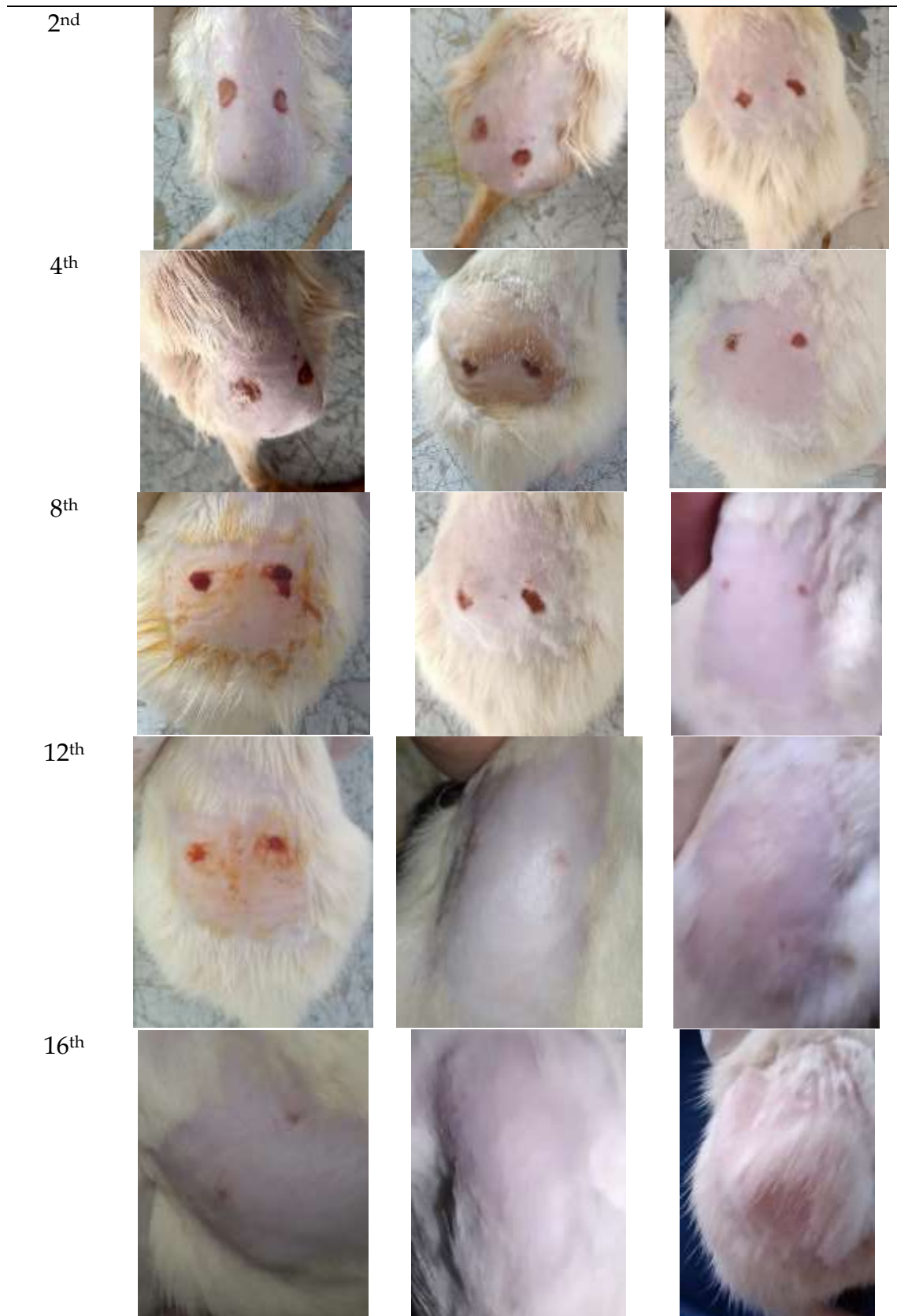


Table 10 shows the wound contraction rate of the rats in their pre-determined days.

## Evaluation of Topical Antibacterial Gel

### 1. Physical Characteristics

The formulated topical antibacterial gel has a brown color, with an appearance that looks similar to a brown ink and has a rummescent odor.

### 2. pH Level

One gram of the formulated topical antibacterial gel was mixed in 100 ml of distilled water. Each of the mixture were labelled as A, B, C, D, and E. The mixtures pH level was measured using a digital pH meter. The digital pH level was first calibrated under a running water before measuring the pH level of the mixtures.

Table 11  
*pH Level of the Gel*

Groups	pH Level
Distilled Water	7.3
A	7.1
B	7.1
C	7.2
D	7.1
E	7.1

The topical antibacterial gel has a pH level of  $7.12 \pm 0.02$ , which indicates that the gel is slightly an alkaline solution but still close to the distilled water.

### 3. Test for thermal stability

The formulated topical antibacterial gel was transferred into five 30 ml vial and was filled 2/3 of the vial. Each vial was covered with cling wrap to prevent it from spilling. It was refrigerated for 2 days under 4-8°C. The formulated gel stored from 4-8 °C became hard but maintained its form. It did not exhibit any phase separation.

Figure 4.  
*The topical antibacterial gel under 4-8 °C*



#### 4. Spreadability

The spreadability of the formulated topical antibacterial gel was measured by putting 1 g of the gel between 2 slides. A weight of 125 g was placed above the 2 glass slides (20x20 cm) for 1 minute. The time needed for it to spread was measured using a phone stopwatch. The length that the gel travelled onto the glass slides were measured using a ruler. This was repeated for three times.

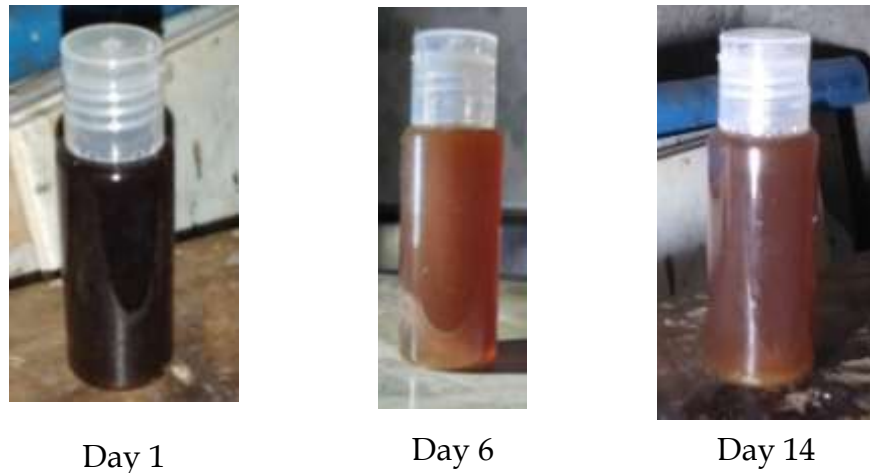
Table 12  
*Spreadability of the Gel*

Groups	Weight (g)	Length of Gel Travelled (cm)	Time Taken (s)	Average Spreadability $S = \frac{M + L}{T}$
1	125	16.36	15	9.424
2	125	16.21	15	9.414
3	125	16.43	15	9.428

#### 5. Phase Separation

The formulated antibacterial gel did not exhibit any phase separation under the dark storage area during its day 1 to day 7 of observation. However, during day 10 to day 14, it exhibited phase separation. The color of gel eventually became lighter, from black-brown to brown. It was also important to note that the formulated topical antibacterial gel became more liquid, losing its semi-solid phase.

Figure 5  
Phase Separation of the Gel



## DISCUSSION

Huh et al. (2010) reported the presence of 10 fatty acids alkyl esters which were evaluated for melanogenesis, supporting the presence of fatty acids in *O. triangularis*. Fatty acid alkyl esters (FAAEs) are a group of neutral lipids naturally occurring in olive oils. They are created through the esterification process, where free fatty acids (FFAs) react with small alcohols to form these compounds (Carmen Perez-Camino et al., 2008). Luo et al. (2022) detected three anthocyanins with the presence of sugar moieties (glucoside, rutinoside and malonyl-glucopyranoside) attached to specific positions of the anthocyanidin molecules. Glycosidic flavonoids are a subclass of flavonoids characterized by the attachment of sugar molecules (glycosides) to the flavonoid aglycone via glycosidic bonds (Kumar & Pandey, 2013). Flavone C-glycosides were also detected from the leaves of *O. triangularis* (Fossen et al., 2005). Flavone C-glycosides are polyphenolic compounds, which means they contain multiple phenolic (hydroxyl) groups in their structures (Miao et al., 2022). Phenolic compounds are known for their antioxidant properties and other biological activities. Additionally, one of the compounds mentioned, apigenin 6-C-(2''-O-(6'''-(E)-p-coumaroylglucoside)- $\beta$ -glucopyranoside), contains a coumaroyl moiety attached to the glucose molecule. Coumarins are a class of aromatic compounds with a benzene ring fused to an  $\alpha$ -pyrone ring (Kaur & Kaur, 2015), and they are known for their diverse biological activities, which could potentially support the presence of coumarins. Yussof et al. (2013) also detected steroids in the plant.

Flavonoids and phenolic compounds are widely recognized for their significant health benefits and antioxidant properties, making them valuable in

the treatment and prevention of various human illnesses. Phenolic compounds exhibit diverse biological activities, contributing to their potential in preventing cancer and other diseases. These activities include their ability to prevent mutations and cancer development, reduce inflammation, and protect against oxidative stress. Additionally, they can enhance programmed cell death, inhibit cell growth, prevent DNA damage, promote cell differentiation, hinder cell migration, and disrupt cell signaling pathways. Bioactive compounds found in plants, such as flavonoids, phenolic compounds, and fatty acids, are well-known for their therapeutic potential against various diseases. These compounds have been documented to exhibit diverse biological effects, including antioxidant and antibacterial properties (Karimi et al., 2015). Many coumarins exhibit various biological properties, including anti-inflammatory, anti-ulcer, anti-tumor, antimicrobial, and anticoagulant activities (Anywar & Muhumuza, 2023). Cedillo-Cortezano et al. (2024) reported that essential oils, flavonoids, and phenolic compounds have wound healing properties. These compounds have also been shown to offer advantageous effects due to their anti-inflammatory, antioxidant, and antibacterial properties. Additionally, they also included those secondary metabolites such as phenolics, fatty acids, as well as glycosides can facilitate the wound-healing process by exerting pharmacological effects on the body. These active ingredients exhibit low toxicity and are efficiently absorbed by the skin barrier. Moreover, they stimulate collagen synthesis and aid in the regeneration of protective cells. Plant steroids primarily combine with sugars to create glycosides. Glucocorticoids are the steroidal agents commonly employed to alleviate inflammatory conditions (Mohammadi-Cheraghabadi & Hazrati, 2023).

Diverse groups of secondary metabolites were detected due to the differences in the polarities of the extract solvent than can cause different bioactive compounds to be attracted and extracted from a plant material (Altermimi et al. 2017). The crude extracts of *O. triangularis* contain various secondary metabolites with distinct polarities or affinities, which are soluble in the extracting solvents employed. This leads to differences in the observed presence or absence of secondary metabolites. Various R<sub>f</sub> values indicate the presence of different secondary metabolites within a single extract, reflecting the polarity of each compound. These calculated R<sub>f</sub> values are instrumental in selecting an optimal extracting solvent for further separation of pure compounds from the extract (Pandey et al., 2013).

Diclofenac sodium is frequently selected as a medication for addressing sudden inflammation and pain. It functions by impeding the cyclooxygenase (COX) pathway, thereby halting the production of prostaglandin and other eicosanoids (Ahmed et al., 2020). Fossen et al. (2005) was able to identify flavone C-glycosides luteolin 6-C-(2''-O-, -xylopyranosyl-, -glucopyranoside). In the study of Khan et al. (2020), luteolin was among the glycosides that demonstrated anti-inflammatory effects by blocking the COX and lipoxygenase (LOX) pathways, which play roles in generating prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), a mediator of

inflammation, from arachidonic acid. Hence, the results imply that *O. triangularis* crude extract has anti-inflammatory properties, but not as effective as the standard drug, diclofenac sodium. Diclofenac sodium has a low IC<sub>50</sub> value than the *O. triangularis* crude extract signifying that it can effectively inhibit the biological process at low concentrations (Berrouet et al., 2020). Consequently, it implies that the drug may exhibit lower systemic toxicity when administered to the patient. Diclofenac sodium's lower IC<sub>50</sub> value compared to the *O. triangularis* crude extract indicates that it's better at reducing the biological response. Potency refers to the concentration of a substance required to produce a certain effect, and in this case, diclofenac sodium achieves a 50% inhibition at a lower concentration compared to the crude extract. Even though the *O. triangularis* crude extract has a higher IC<sub>50</sub> value than the standard drug, it still fights inflammation effectively since its IC<sub>50</sub> value is close to the standard drug.

Phenols, which were detected as one of its secondary metabolites, often work alongside NSAIDs and can hinder pro-inflammatory mediators like COX (Rahman, et al., 2022). They can also influence antioxidant pathways by affecting nuclear factor- $\kappa$ B (NF- $\kappa$ B) or nuclear factor-erythroid factor 2-related factor 2 (Nrf-2). Additionally, *O. triangularis* is rich in anthocyanins, which is a phenolic compound that are typically represented by glycosides. The structure of these compounds plays a significant role in their anti-inflammatory effects.

Kim et al. (2018) reported that extracts of *O. triangularis* showed antimicrobial activity in acidic pH against broad spectrum of bacteria including *E. coli* and *S. aureus*. In addition, they found that oxalic acid or oxalate compounds were responsible to the antibacterial activity that was shown in the concentration of pure oxalic acid isolated from the *O. triangularis*. Several detected secondary metabolites such as glycosidic flavonoids, phenols, fatty acids and coumarins also contain antibacterial activities (Tungmunnithum et al., 2018).

Based on the discovery of the natural antibacterial effect derived from *O. triangularis*, it can be used for pharmaceutical advancements, as well as prevention and treatment for food poisoning and diseases caused by the opportunistic pathogens.

MIC is the lowest concentration of an antibacterial agent expressed in mg/L ( $\mu$ g/mL) which, under strictly controlled in vitro conditions, completely prevents visible growth of the test strain of an organism. The lower the MIC value, the less drug is required to inhibit the growth of an organism. Both the test microorganisms were inhibited at small concentrations, implying that *O. triangularis* crude extract are more effective antibacterial agents at lower concentrations.

MIC evaluations are useful during the research and development phase of a product to determine the appropriate concentrations required in the final product. However, it is imperative to recognize that the inhibition of bacterial

growth does not invariably signify the eradication of the bacteria (Finberg *et al.*, 2004).

The concentration of 15-10 µg/ml of crude extract against *E. coli* was only able to inhibit, but not completely eradicate. Each well tested against *S. aureus* had indicated growth when cultured in MHA for 24 hours. This imply that *O. triangularis* crude extract may exhibit bacteriostatic activity against *S. aureus*. Bacteriostatic agents can be disinfectants, antiseptics, preservatives, or bacteriostatic antibiotics. Bacteriostatic antibiotics are medications that stop bacterial cellular activity without killing the bacteria directly (Loree & Lappin, 2023). They do this by stalling bacterial cellular activity, and are used to treat bacterial infections.

Ten grams of CMC was weighed in a digital balance and was transferred in a beaker. Twenty milliliter of distilled water was poured gradually into the CMC until all of the distilled water was transferred into the beaker. Hot distilled water was poured into the 10 g gelatin. After the gelatin was dissolved, it was gradually added into the CMC solution and was continuously stirred until it became homogenous. Five milliliters (ml) of *O. triangularis* crude extract were added to the CMC-Gelatin solution while continuously stirring. 50 ml of water was added to the mixture to achieve the desired 100 ml. The final concentration of the product was 5 µg/ml.

The rats did not exhibit any erythema or edema for the past three days. Rescober *et al.* (2022) reported that CMC-Gelatin solution did exhibit some wound healing property but not as significant as the plant extract. The rat treated with povidone-iodine, exhibited the slowest wound contraction. Povidone iodine is an effective antiseptic that does not impede wound healing (Bigliardi *et al.*, 2017). Additionally, iodine is an effective antiseptic agent that shows neither the purported harmful effects nor a delay of the wound-healing process, particularly in chronic and burn wounds (Vermeulen *et al.*, 2010).

The shamrock topical antibacterial gel has a brown color, with an appearance that looks similar to a brown ink and has a rummescent odor. It has a pH level of  $7.12 \pm 0.02$  and a spreadability of  $9.422 \pm 0.004163332$ . The shamrock topical antibacterial gel was thermally stable under 4-8°C. It also did not exhibit any phase separation under the dark storage area during its day 1 to day 7 of observation but exhibited phase separation on day 10 to day 14.

## CONCLUSIONS AND RECOMMENDATIONS

Six secondary metabolites were detected in the crude extract of *O. triangularis* using the solvent, 80% ethyl alcohol and solvent system, ethyl acetate-hexane (1:1) which were essential oils, phenols, fatty acids, coumarins, glycosidic flavonoids, and steroids. Additionally, *O. triangularis* crude extract have anti-

inflammatory properties but not as effective as the standard drug, diclofenac sodium.

With a concentration of 1 µg/ml, the crude extract of *O. triangularis* exhibited maximum relative percentage inhibition against *E. coli* (83.72%) followed by a concentration of 0.1 µg/ml with a relative percentage inhibition of 74.46%. The minimum relative percentage inhibition was observed in case of the crude extract against *S. aureus* (2.77%). The MIC of *O. triangularis* against *E. coli* was found to be effective at 15-3 µg/ml. The MIC of *O. triangularis* against *S. aureus* was found to be effective at 10-1 µg/ml. For the MBC, only 5-3 µg/ml of the crude extract against *E. coli* was found to completely inhibit and eradicate its growth. The crude extract exhibited bactericidal activity against *E.coli* while the crude extract against *S. aureus* has only exhibited bacteriostatic activity.

The rats did not exhibit any erythema or edema for the past three days. The rat treated with the *O. triangularis* healed faster than the rats with the hydrogel and povidone-iodine. Rescober *et al.* (2022) reported that CMC-Gelatin solution did exhibit some wound healing property but not as significant as the plant extract. The rat treated with povidone-iodine, exhibited the slowest.

The shamrock topical antibacterial gel has a brown color, with an appearance that looks similar to a brown ink and has a rummescent odor. It has a pH level of  $7.12 \pm 0.02$  and a spreadability of  $9.422 \pm 0.004163332$ . The shamrock topical antibacterial gel was thermally stable under 4-8°C. It also did not exhibit any phase separation under the dark storage area during its day 1 to day 7 of observation but exhibited phase separation on day 10 to day 14.

## FURTHER STUDY

1. The researcher would like to recommend to future researchers to use different extracting solvents, their concentrations, and compare their percent yield as well as use different solvent systems to extract different secondary metabolites.
2. *O. triangularis* crude extract effectively eradicated *E. coli* and was therefore found to be bactericidal. It is recommended that further pharmaceutical advancements such as turning the *O. triangularis* crude extract as a capsule or liquid drink to assess *E. coli* infecting diseases. The shamrock topical antibacterial gel is recommended to undergo antibacterial assay to different test microorganisms, especially the Gram-negative microorganisms.
3. *O. triangularis* crude extract is recommended to be subjected in different bioassays including DPPH free radical scavenging assay to assess its antioxidant property; cytotoxicity test to evaluate its anti-cancer property; *in vivo* anti-inflammatory assay using carrageenan-induced rats; anti-ulcer, antidiuretic and anticoagulant test.
4. It is also recommended to assess the wound healing activity of *O. triangularis* using histological examination to further provide detailed

information about tissue morphology, cellular dynamics, extracellular matrix remodeling, and vascularization. Researchers may also conduct chick embryo chorioallantoic membrane (CAM) assay to evaluate further angiogenesis, tumor research and tissue engineering.

## ACKNOWLEDGMENT

The researcher would like to thank her parents, Juanito M. Dupra, Jr. and Amelia B. Dupra, for the unconditional support that they had given. Special thanks to Ms. Ysha Gleshi V. Reynon, for her love and encouragement.

## REFERENCES

- Abu Bakar, M., Haque, M., McKimm, J., & Sartelli, M. (2019). Health Care-Associated Infections-An Overview. *Infection and Drug Resistance*. 2018; 11:2321-2333. doi: 10.2147/IDR.S177247
- Ahmed, T., Archie, S.R., Faruk, A., Chowdhury, F.A., Shoyab, A., Ahsan, C.R. (2020). Evaluation of the Anti-Inflammatory Activities of Diclofenac Sodium, Prednisolone and Atorvastatin in Combination with Ascorbic Acid. *Antiinflamm Antiallergy Agents Med Chem*. 2020 Sep; 19(3): 291-301. Published online 2020 Sep. doi: 10.2174/1871523018666190514112048
- Alfaro, R.A. & Davis, D.D. (2023). Diclofenac. National Library of Medicine. [ncbi.nlm.gov/books/NBK557879](https://pubmed.ncbi.nlm.gov/books/NBK557879)
- Altermimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G., & Lightfoot, D. (2017). Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants (Basel)*. 2017 Dec; 6(4): 42. Published online 2017 Sep 22. doi: 10.3390/plants6040042
- Anywar, G. & Muhumuza, E. (2023). Bioactivity and toxicity of coumarins from African medicinal plants. *Front. Pharmacol*. Volume 14 - 2023. <https://doi.org/10.3389/fphar.2023.1231006>
- Arakareddy, B., Johnson, B.L., & Sam, K.K. (2020). Formulation of an Anti-Bacterial Cream from Plant Oxalis corniculata and its Evaluation. *International Journal of Current Pharmaceutical Research*. Vol. 12, Issue 5, 2020. doi: 10.22159/ijcpr.2020v12i5.39763
- Arancel, F. & Rovillos, K. (2019). Healthcare-Associated Infections among Patients in a Surgery Ward: Cross-Sectional Study. *Southern Philippines Medical Center (SPMC) J Health Care Serv*. 2019;6(1):4. [http://n2t.net/ark/76951/jhcs94gn2f](http://n2t.net/ark:/76951/jhcs94gn2f)
- Arakelyan, S. Arakelyan, H. (2020) Beauty for Health-Oxalis triangularis. [https://www.researchgate.net/publication/340829095\\_Beauty\\_For\\_Health\\_Oxalis\\_Triangularis/link/5ea00b23a6fdccda592c34cd/download?\\_tp=eyJjb250ZXh0Ijp7ImZpcnN0UGFnZSI6InB1YmxpY2F0aW9uIn19](https://www.researchgate.net/publication/340829095_Beauty_For_Health_Oxalis_Triangularis/link/5ea00b23a6fdccda592c34cd/download?_tp=eyJjb250ZXh0Ijp7ImZpcnN0UGFnZSI6InB1YmxpY2F0aW9uIn19)
- Aykul, S., & Martinez-Hackert, E. (2016). Determination of half-maximal inhibitory concentration using biosensor-based protein interaction analysis.

- Analytical biochemistry, 508, 97-103.  
<https://doi.org/10.1016/j.ab.2016.06.025>
- Bandyopadhyay, D. (2021). Topical Antibacterials in Dermatology. *Indian Journal of Dermatology*. 2021 Mar-Apr; 66(2): 117-125. doi: 10.4103/ijd.IJD 99 18
- Barrasa, A., Henarejos, V., Merck, B., O'Connor, Pastor, C., Puyana, J.C., & Villalonga, A. (2022) Implementing a mHealth-Based Patient and Nurse Educational Program to Reduce Wound Infection in Rural Philippines. *Annals of Global Health*. 2022; 88(1):76. doi: 10.5334/aogh.3834
- Belkaid, Y., Byrd, A.L., & Segre, J.A. (2018) The Human Skin Microbiome. *Nature Reviews Microbiology*. Vol. 16, 143-155 (2018).  
<https://doi.org/10.1038/nrmicro.2017.157>
- Berrouet, C., Dorilas, N., Rejniak, K. A., & Tuncer, N. (2020). Comparison of Drug Inhibitory Effects (IC50) in Monolayer and Spheroid Cultures. *Bull Math Biol*. 2020 Jun 3; 82(6): 68. Published online 2020 Jun 3. doi: 10.1007/s11538-020-00746-7
- Bessa, L.J., Fazii, P., Di Giulio, M., & Celini, L. (2015) Bacterial Isoclates from Infected Wounds and their Antibiotic Susceptibility Patterns: Some Remarks about Wound Infection. *International Wound Journal*. Vol. 12 No. 1, pg.47-52. doi:10.1111/iwj.12049
- Bigliardi, P.L., Alsagoff, S.A.L., El-Kafrawi, H.Y., Pyon, J. Wa, C.T.C., & Villa, M.A. (2017). Povidone iodine in wound healing: A review of current concepts and practices. *International Journal of Surgery*. Volume 44, August 2017, Pages 260-268. <https://doi.org/10.1016/j.ijssu.2017.06.073>
- Carmen Perez-Camino, M. Cert, A., Romero-Segura, A., Cert-Trujillo, R. & Moreda, W. (2008). Alkyl Esters of Fatty Acids a Useful Tool to Detect Soft Deodorized Olive Oils. *Journal of Agricultural and Food Chemistry*. 2006,56,15,6740-6744. <https://doi.org/10.1021/jf801131b>
- Carmona-Cruz, S., Orozco-Covarrubias, L., & Saez-de-Ocariz, M. (2022) The Human Skin Microbiome on Selected Cutaneous Diseases. *Frontiers*. Vol. 12 (2022). doi: 10.3389/fcimb.2022.834135
- Cedillo-Cortezano, M., Martinez-Cuevas, L.R., Marquez-Lopez, J.A., Barrera-Lopez, I.L., Escutia-Perez, S. & Petricevich, V.L. (2024) Use of Medicinal Plants in the Process of Wound Healing: A Literature Review. *Pharmaceuticals* 17(3):303 doi: 10.3390/ph17030303
- Chaiya, P., Senarat, S., Phaechamud, T., & Narakornwit, W. (2022, January 1). In vitro anti-inflammatory activity using thermally inhibiting protein denaturation of egg albumin and antimicrobial activities of some organic solvents. *Materials Today: Proceedings*; Elsevier BV. <https://doi.org/10.1016/j.matpr.2022.04.916>
- Dons, T. & Soosairaj, S. (2018). Evaluation of Wound Healing Effect of Herbal Lotion in Albino Rats and its Antibacterial Activities. *Clinical Phytoscience*. Vol 4, 6 (2018). doi: 10.1186/s4086-0180-0065-z
- European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMD) (2003). Determination of Minimum Inhibitory Concentrations (MICs) of the

- Antibacterial Agents by Broth Dilution. EUCAST Discussion Document E.Dis 5.1.
- Finberg, R. W., Moellering, R. C., Tally, F. P., Craig, W. A., Pankey, G. A., Dellinger, E. P., West, M. A., Joshi, M., Linden, P. K., Rolston, K. V., Rotschafer, J. C., & Rybak, M. J. (2004). The importance of bactericidal drugs: future directions in infectious disease. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 39(9), 1314–1320. <https://doi.org/10.1086/425009>
- Fossen, T., Rayyan, S., Holmberg, M.Y., Nateland, H.S., Andersen, O.M. (2005). Acylated Anthocyanins from Leaves of *Oxalis triangularis*. *Phytochemistry*. Vol. 66, May 2005, pg. 1133-1140. doi: 10.1016/j.phytochem.2005.04.009
- Fossen, T., Rayyan, S., Holmberg, M.H., Nimtz, M. & Andersen, O.M. (2005). Covalent Anthocyanin-Flavone Dimer from Leaves of *Oxalis triangularis*. *Phytochemistry*. 68 (5), 652-662, 2007. doi: 10.1016/j.phytochem.2006.10.030
- Guevarra, B.Q., Claustro, A.L., Espeso, E.I., ... & Ysrael, M.C. (2005). A Guidebook to Plant Screening: Phytochemical and Biological. Research Center for Natural Sciences, University of Santo Tomas, UST. ISBN: 971-506-263-6
- Huh, S., Kim, Y. S., Jung, E., Lim, J., Jung, K. S., Kim, M. O., Lee, J., & Park, D. (2010). Melanogenesis inhibitory effect of fatty acid alkyl esters isolated from *Oxalis triangularis*. *Biological & pharmaceutical bulletin*, 33(7), 1242–1245. <https://doi.org/10.1248/bpb.33.1242>
- Khan, H., Pervaiz, A., Intagliata, S., Das, N., Venkata, K.C.N., Atanasov, A.G., Najda, A., Nabavi, S.M., Wang, D., Pittala, V., Bishayee, A. (2020). DARU Journal of Pharmaceutical Sciences. 2020 Jun; 28(1): 387–401. Published online 2020 Feb 14. doi: 10.1007/s40199-019-00319-7
- Kaur, J. & Kaur, G. (2015). An insight into the role of citrus bioactives in modulation of colon cancer. *Journal of Functional Foods*. Vol. 13, pg. 239-261. <https://doi.org/10.1016/j.jff.2014.12.043>
- Kim, H.S., Kim, J.S., Kim, Y. G. & Ko, D.Y. (2018). Extracts of *Oxalis triangularis* shows Broad Spectrum Antibacterial Activity in Acidic Condition. *Biomedical Research*. Vol. 29, No. 20. (2018). doi: 10.4066/biomedicalresearch.29-18-1089
- Karimi, E., Jaafar, H.Z., Ghasemzadeh, A. et al. Fatty acid composition, antioxidant and antibacterial properties of the microwave aqueous extract of three varieties of *Labisia pumila* Benth. *biol res* 48, 9 (2015). <https://doi.org/10.1186/0717-6287-48-9>
- Kumar, S. & Pandey, A.K. (2013). Chemistry and Biological Activities of Flavonoids: An Overview. *Scientific World Journal*. 2013; 2013: 162750. Published online 2013 Dec 29. doi: 10.1155/2013/162750
- Lipatan, M.C., Macalindong, S.S. & Ramirez, A.D. (2022). Surgical Site Infection (SSI) Surveillance Program for Mastectomy in the Department of Surgery of the University of the Philippines-Philippine General Hospital. *Acta Medica Philippina*. 56(6). doi: 10.47895/amp.v56i6.3586
- Loree, J., & Lappin, S.L. (2023). Bacteriostatic Antibiotics. StatPearls. <https://www.ncbi.nlm.nih.gov/books/NBK547678/>

- Luber, P., Nartelt, E., Genschow, E. Wagner, J. & Hahn, H. (2003). Comparison of Broth Microdilution, E Test and Agar Dilution Methods for Antibiotic Susceptibility Testing of *Campylobacter jejuni* and *Campylobacter coli*. *Journal of Clinical Microbiology*. 2003 Mar; 41(3): 1062-1068. doi: 10.128/JCM.41.3.1062-1068.2003
- Luo, B., Chen, L., Chen, G., Wang, Y., Xie, Q., Chen, X., & Hu, Z. (2022). Transcription and Metabolism Pathways of Anthocyanin in Purple Shamrock (*Oxalis triangularis* A.St.-Hil.). *Metabolites*, 12(12), 1290. <https://doi.org/10.3390/metabo12121290>
- Madhungara, H.D.T. & Samarakoon, N.A. (2023). In Vitro Anti-Inflammatory Egg Albumin Denaturation Assay: Methodology. *ResearchGate*. doi: 10.3140./RG.2.2.22740.88960
- Maskarinec, S.A., Sharma-Kuinkel, B.K., Turner, N.A. (2019). *Staphylococcus aureus*: An Overview of Basic and Clinical Research. *National Review of Microbiology*. 2019; 17:203-218. doi:10.1038/s41579-018-0147-4
- Mohammadi-Cheraghbabadi, M. & Hazrati, S. (2023). Chapter 5 Terpenoids, steroids, and phenolic compounds of medicinal plants. In C. Arora, D. Verma, J. Aslam & P. Mahish (Ed.), *Phytochemicals in Medicinal Plants: Biodiversity, Bioactivity and Drug Discovery* (pp. 105-130). Berlin, Boston: De Gruyter. <https://doi.org/10.1515/9783110791891-005>
- Miao, L., Zhang, H., Yang, L., Chen, L., Xie, Y., and Xiao, J. (2022) Chapter 4.8-Flavonoids. *Antioxidants Effects in Health*. 2022, pg. 353-374. <https://doi.org/10.1016/B978-0-12-819096-8.00048-3>
- National Nutrition Council. (2021). Diet and Acne. <https://nnc.gov.ph/regional-offices/mindanao/region-xi-davao-region/6533-diet-and-acne/>
- Pandey, P., Mehta, R., Upadhyay, R. (2013). Physico-chemical and preliminary phytochemical screening of *Psoralea corylifolia*. *Archives of Applied Science Research*. 5(2): 261-265.
- Poklar, N., & Vesnaver, G. (2000). Thermal Denaturation of Proteins Studied by UV Spectroscopy. *Journal of Chemistry and Education*. 2000, 77, 3, 380. doi: 10.1021/ed077p380
- PSIBERG (2022). Rf Values: Definition, Calculation and Explanation. <https://psiberg.com/rf-values/>
- Rahman, M.M., Rahaman, M.S., Islam, M.R., ... & Uddin, M.S. (2022). Role of Phenolic Compounds in Human Disease: Current Knowledge and Future Prospects. *Molecules*. 2022 Jan; 27(1): 233. Published online 2021 Dec 30. doi: 10.3390/molecules27010233
- Rescober, A.A., Blanco, I.F., Custudio, S.E., Daguman, F.L., Dollesin, J., Janio, J.A., & Viray, J.M. (2022). Antimicrobial and Wound Healing Activity of *Ixora coccinea* Leaf Extract in Hydrogel Formulation. *Acta Medica Philippina*. Vol. 56, No. 7
- Sotocinal, S. G., Sorge, R. E., Zaloum, A., Tuttle, A. H., Martin, L. J., Wieskopf, J. S., Mapplebeck, J. C., Wei, P., Zhan, S., Zhang, S., McDougall, J. J., King, O. D., & Mogil, J. S. (2011). The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Molecular pain*, 7, 55. <https://doi.org/10.1186/1744-8069-7-55>

- Tazeze, H., Mequanente, S., Nigussie, D., Legesse, B. A., Makonnen, E., & Ayele, T. M. (2021, October 1). Investigation of Wound Healing and Anti-Inflammatory Activities of Leaf Gel of *Aloe trigonantha* L.C. Leach in Rats. *Journal of Inflammation Research*; Dove Medical Press. <https://doi.org/10.2147/jir.s339289>
- Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines (Basel)*. 2018 Sep; 5(3): 93. Published online 2018 Aug 25. doi: 10.3390/medicines5030093.
- Vermeulen, H., Westerbos, S. J., & Ubbink, D. T. (2010). Benefit and harm of iodine in wound care: a systematic review. *The Journal of hospital infection*, 76(3), 191-199. <https://doi.org/10.1016/j.jhin.2010.04.026>
- Yalanis, G. C. (2022). Topical antibiotics: Uses, Common Brands, and Safety Info. <https://www.singlecare.com/drug-classes/topical-antibiotics>
- Yussof, A. I. M. (2013). Tissue Culture, Biological Activities and In Vitro Flowering of *Oxalis triangularis*. University of Malaya. : [https://www.scholar.google.com.ph/scholar?cluster=13610462395727167726&hl=en&as\\_sdt=0,5#d=gs\\_qabs&t=1697859751966&u=#p=ys6MnVk2A48J](https://www.scholar.google.com.ph/scholar?cluster=13610462395727167726&hl=en&as_sdt=0,5#d=gs_qabs&t=1697859751966&u=#p=ys6MnVk2A48J)
- Zarai, Z., Kadri, A., Chobba, I.B., Mansour, R.B., Bekir, A., Mejdoub, H. & Gharsallah, N. (2011) The in-vitro Evaluation of Antibacterial, Antifungal and Cytotoxic Properties of *Marrubium vulgare* L. essential oil grown in Tunisia. *Lipids in Health and Disease*. 10, 161 (2011). doi: 10.1186/1476-511X-10-16Bad