Wound Healing Activity of Herbal Ointment Containing the Ethanolic Leaf Extract of Gumamela (Hibiscus rosa-sinensis)

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Abstract: The wound healing process is a multi-step cellular and biochemical process. For wound healing, antibiotics; preservatives; desalination agents; chemicals; and others are used. Some of these synthetic drugs are limited due to side effects. For this reason, the use of medicinal plants for wound healing has increased in popularity over the years due to the reduction of side effects. In fact, previous studies proved that Hibiscus rosa-sinensis (gumamela) extract can be used to treat wounds. Thus, the purpose of this study intends to evaluate the wound healing potentials of the formulated herbal ointment containing the gumamela ethanolic leaf extracts. The gumamela leaf ethanolic extract was mixed into the ointment foundation. The herbal ointment was then formulated. For the wound healing study, untreated and gumamela ointment treated wounds of albino mouse were observed to have a comparison after the experimentation. The physicochemical parameters of gumamela ointment including color, odor, washability, solubility, consistency, and pH level were all evaluated, and the results were satisfactory. Throughout the experimental period, there was a larger wound closure percentage in the wound treated with gumamela ointment as compared to the untreated. Hence, this finding clearly indicates that the ointment containing the gumamela leaf extract can enhance the healing of a wound as indicated by improved rates of wound closure.

Key Words: hibiscus rosa-sinensis; gumamela ointment; wound healing activity; wound splint model; ethanolic leaf extract

1. INTRODUCTION

According to Farahpour et al. (2019), the wound healing process is known as a cellular and biochemical interdependent step aimed at wound healing. Many individual herbal & multi-herbal compositions have been reported to accelerate wound healing in wound models. In fact, it has been scientifically proven that gumamela is used for wound healing (Shen et al., 2017). It is an ornamental plant that grows in China as an evergreen herb. It's only form, consisting of five red petals, is an ornamental mallow shrub from East Asia (Kitayima et al., 2010).

1.1. Theoretical Framework

As contested by Boateng et al. (2008), for wound healing; antibiotics, preservatives, desalination agents, chemicals and others are used. Some of these synthetic drugs are limited due to side effects. For this reason, the use of medicinal plants for wound healing has increased in popularity over the years due to the reduction of side effects and wound care (Farahpour et al., 2019). According to the findings of Al-Snafi et al. (2018), gumamela extract may be used to treat wounds. Kumar et al. (2012) claimed that this plant has various pharmacological activities that can be used in various medical applications. For this reason, this study intends to validate the potential of the newly developed wound healing ointment containing the gumamela leaf extract.

1.2. Research Questions

What are the physicochemical parameters necessary in the evaluation of gumamela herbal ointment?

Does the gumamela herbal ointment show potential in healing wounds?

1.3. Scope and Delimitation

This study will focus on evaluating the wound healing potential of the formulated herbal ointment containing gumamela ethanolic leaf extracts. The objective of this study is limited since the other possible medicinal properties including the antibacterial activity of the ointment will not be studied. This study is also limited due to the use of a small sample size (1 albino mouse). Moreover, there was no positive control used in the treated and controlled experiment.

2. METHODOLOGY

Experimental research design is motivated by hypotheses, and statistical analysis is used to confirm or disprove a theory (Nunmaker et al., 2001). It is the most precise type of experimental design and can be performed on at least two randomly assigned dependent subjects with or without a pretest. The researchers used posttest only control design. The posttest-only control design is a study in which at least two groups are used, one of which does not receive a treatment or intervention, and data on the outcome measure is obtained after the treatment or intervention. The researchers used 1 albino male mouse. The untreated and gumamela ointment treated wounds of the mouse were observed to have a comparison after the experimentation.

2.1. Sampling Procedure

The researchers obtained a 27g 1 male albino mouse (Mus musculus) from the pet shop located at Dolores, Taytay, Rizal. albino rats were used for this study because it represents a cost-effective animal model that is easy to genetically modify for mechanistic research.

2.2. Ethical Consideration

To ensure the safety of the subject (albino mouse) the researchers seek technical assistance from a professional veterinary doctor to do the excision wounds on albino mouse. The researchers also assure that in line with this activity, precautionary actions were considered such as taking care of the subject with the intention that no subject will be exterminated. Moreover, proper handling protocols were observed for the safety of the researchers from getting bitten by the subject.

2.3. Proposed Product

Figure 1
Formulated gumamela ointment

2.4. Procedures

2.4.1. Preparation of gumamela Leaf Ethanolic Extract

Gumamela leaves were collected and thoroughly washed with distilled water to clean the adhering dust particles. After collecting and washing, the leaves were dried under the shade until they dried. The dried leaves were ground into powder with the aid of an electric blender. Thereafter, the 100g powder was imbibed with 350ml of 90% ethanol for 3 hours and moved to an airtight container of 150ml 90% ethanol for 7 days of maceration with occasional stirring. Finally, the Ethanolic extract of gumamela leaves was collected and condensed to produce a blackish green residue, which was then filtered using the simple filtration process. The extract was kept in an airtight container in a cold, dark place.

2.4.2. The procedure for making herbal ointment is as follows:

A. To make the ointment base, weigh precisely grated hard paraffin wax (25g) and place it in an evaporating dish over a water bath. The other ingredients (50g of petroleum jelly and 20ml of coconut oil) was added after the hard paraffin had melted, and gently stirred to aid melting and homogeneous mixing until the ointment base had cooled.
B. To make gumamela ointment, weigh the gumamela ethanolic leaf extract (5ml) and blend it into the ointment base to make a smooth paste. Gradually add more base until the ointment is homogeneous.

2.4.3. The following physicochemical parameters were used for the evaluation of the ointment:

Color & odor
Visual inspection was used to check the color and odor of the prepared ointment.
pH
The pH of ointment was determined using pH paper.
Solubility
The ointment was observed if it is soluble in water and alcohol.
Washability
After applying the ointment formulation to the skin, the degree to which it could be washed away with water was determined.
Consistency
Smooth and no greediness were observed.

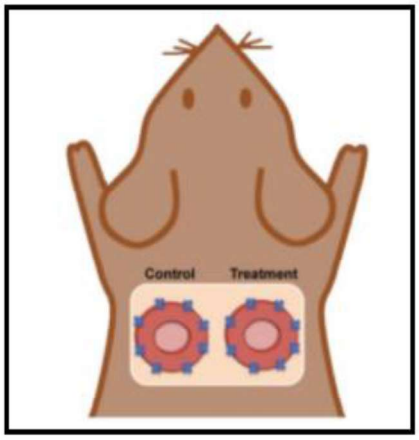
2.4.4. Excisional wound splinting procedure:

Wound healing activities were evaluated using the mouse excisional wound splinting model. The albino mouse was anesthetized prior to creation of the wounds. Subcutaneous injection of 50 mg/ml intravenous Tiletamine-Zolazepam was used. The dorsal fur of the animal was shaved using a razor

blade. Two excision wounds were made using a biopsy punch to cut away a 6mm diameter length full thickness of skin from the dorsal area. Two silicon discs were adhered to the skin around the wound and sutured in place, preventing local skin contraction. After suturing, the wounds were covered with bandages. The animal was placed in a clean plastic cage. The wounds of the mouse were treated topically and categorized as untreated wound and wound with gumamela ointment. The wound area was measured on the first, 6th, 8th, 10th, & 12th day post-surgery.

2.5. Experimental model

Figure 2
Excisional wound splinting model



The researchers used the excisional wound splinting wound healing model in albino mouse (Mus musculus). Two full-thickness wounds are produced on either side of the mouse's dorsal region in this model. Silicone splints are adhered and sutured to the wound's perimeter, creating a human-like model (Dunn et al., 2013). According to Wang et al., (2013), Mouse' wounds heal differently to humans, primarily due to the process of contraction. This is in part, due to an extensive subcutaneous striated muscle layer called the 'panniculus carnosus' that is largely absent in humans. In mice, this muscle layer allows the skin to move independently of the deeper muscles and is responsible for the rapid contraction of skin following wounding. To overcome this limitation, the researchers used the excisional wound splinting wound healing model in albino mouse. The use of silicone splints is to allow the re-epithelialization and new tissue formation, a key feature of this model, which is analogous to what happens in humans.

2.6. Data Analysis Procedure

For the wound healing study, the albino mouse was wounded as part of the experimentation. Two wounds were characterized as treated (wound with gumamela ointment) and negative controlled (wound with no treatment). The untreated and treated wounds were observed to have a comparison after the experimentation.

2.7. Instruments

Between the first day and 12 post-surgery, the researchers used an observation sheet to record the untreated and treated wound areas of the albino mice. To determine the wound area: the diameter (mm) of each wound was measured and was then computed using the formula of πr^2. After that, the percentage of wound closure was computed as follows: (area of original wound – area of current wound) / area of original wound x 100$\pi r^2\pi r$

3. RESULTS AND DISCUSSION

3.3. What are the physicochemical parameters necessary in the evaluation of gumamela herbal ointment?

The following physicochemical parameters were used for the evaluations of the ointment:

Table 1
Physicochemical evaluation of the formulated gumamela ointment

Physicochemical parameters	Observations
1. color	Ocado green
2. odor	Characteristic
3. pH level	pH level of 5
4. consistency	Smooth
5. washability	Good
6. solubility	Soluble in water and alcohol

Physicochemical parameters including color, odor, washability, solubility, consistency, and pH level were all evaluated, and the results were satisfactory.

Color & odor

Visual inspection was used to check the color and odor of the prepared ointment.

pH level

The pH level of ointment was determined using pH paper. A little amount of ointment was wiped on the pH strip. The paper turned into a color yellow orange which indicates a pH level of 5.

Solubility

The ointment was observed if it is soluble in water and alcohol.

Washability

After applying the ointment formulation to the skin, the degree to which it could be washed away with water was determined.

Consistency

Smooth and no greediness were observed

3.2. Does the gumamela herbal ointment show potential in healing wounds?

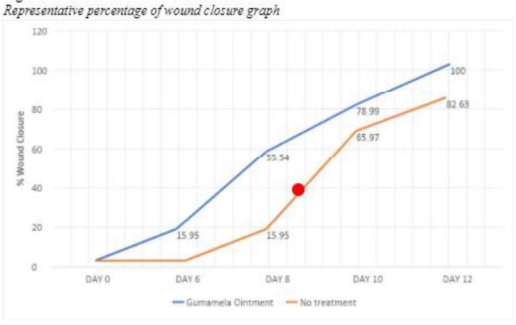
In this finding, the researchers presented table and figures which show the observed albino mouse's wound area and wound closure percentage from day 0, day 6, day 8, day 10 and day 12. They are presented in order to have a comparison between the wound with gumamela ointment and the untreated.

Table 2
Wound area of the treated and untreated wound

	Wound Area (mm^2)				
	DAY 0	DAY 6	DAY 8	DAY 10	DAY 12
Treated (gumamela Ointment)	28.27 mm^2	23.76 mm^2	12.57mm^2	5.94mm^2	0 mm^2
Untreated	28.27 mm^2	28.27 mm^2	23.76 mm^2	9.62mm^2	4.91 mm^2

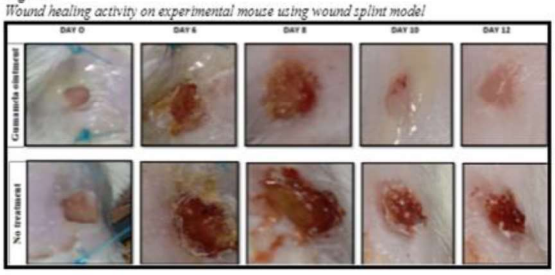
Throughout the experimental period, there was a larger decrease in the area of the wound treated with gumamela ointment as compared to the untreated wound.

Figure 3
Representative percentage of wound closure graph



The researchers calculated (area of original wound – area of current wound) / area of original wound x 100 as the percentage of wound closure (Wang et al., 2013). There was a day-by-day increase in the wound closure percentage of the untreated and wound treated with gumamela ointment. However, the untreated wound closure percentage was less remarkable as compared to the wound closure percentage of gumamela ointment treated wound. The graph shows that by the 10th day of post-surgery, 78.99% of wound closure was observed on the wound with gumamela ointment while the untreated wound had only 65.97% of wound closure. Hence, the findings of this study clearly indicate that the ointment containing gumamela leaf extract can enhance the closure of a wound.

Figure 4
Wound healing activity on experimental mouse using wound splint model



Throughout the experimental phase, the two wounds showed a day-by-day wound closure. However, the wounds treated with gumamela ointment were observed to show a more significant increase in wound healing activity as compared with untreated wounds. For the wound treated with gumamela ointment, a sharp decrease in the wound area was observed between the 6th & 12th after surgery, while a gradual reduction in the untreated wound area was recorded between days 6 & 12. The changes in the wound area as measured on days 0, 6, 8, 10, & 12 post surgery are shown in Table 2. Hence, this finding clearly indicates that the ointment containing the gumamela leaf extract can enhance the healing of a wound as indicated by improved rate wound closure.

Wound healing is a normal process in which dermal and epidermal tissues regenerate. When a wound occurs, a series of simultaneous events occur to repair the injury (Iba et al., 2004). There are three stages of these processes: inflammatory, proliferative, and remodeling (Stadelmann et al., 1998). In the inflammatory phase, bacteria and debris are phagocytosed and removed, while in the proliferative phase, cytokines and mediators are released, triggering cell migration and division. Angiogenesis, collagen deposition, granulation tissue development, epithelialization, and wound contraction are all part of the proliferative method (Midwood et al., 2004). During epithelialization, epithelial cells crawl across the wound bed to cover it (Garg, 2000).

4. CONCLUSIONS

The ethanolic leaf extract of gumamela was tested for wound healing operation. When the extract was mixed into the ointment foundation, the activity was preserved for topical use in the treatment of wounds. Its physicochemical parameters including color, odor, washability, solubility, consistency, and pH level were all evaluated, and the results were satisfactory.

Throughout the experimental period, there was a larger wound closure percentage in the wound treated with gumamela ointment as compared to the untreated one. Hence, this finding clearly indicates

that the ointment containing the gumamela leaf extract can enhance the healing of a wound as indicated by improved rates of wound closure.

4.1 Recommendations

The researchers would like to advise potential researchers who would use this study as a guide to use a larger sample size (more than two albino mice as an experimental model) because using a larger sample size in an experiment would make it less likely to draw an incorrect conclusion. They could also conduct a positive control on their experiment in order to obtain more reliable results.

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