

Histologic Property Of *Psidium Guajava* Linn Guava Leaf Extract In Wound

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Abstract: *A wound is a break in the continuity of the skin. The body's response to injury and the restoration of the same is healing. Wound healing is a biological process that is initiated by trauma and often terminated by scar formation. In this research, guava leaf extract was used as antiseptic and its efficacy was tested to commercially available products. The result revealed that all mice which received guava leaf extract formed scar earlier as compared to povidone iodine and PNSS. Since maturation phase which also refers to remodeling phase is responsible for the new epithelium and final scar tissue formation, and as the development of these completes the complex process of wound healing, the researchers suggest that the use of guava leaf extract in comparison with povidone iodine and PNSS when it comes to wound healing is the most effective treatment in promoting normal and more rapid wound healing.*

Keywords: *histologic, wound, guava, leaf extract*

1. INTRODUCTION

Healing of wound is a sign of growth and an important biological phenomenon. Whether acute or chronic, wounds can compromise an individual's wellbeing, self-image, workability and freedom. Skin wounds affect the quality of life of patients significantly (Research center for traditional medicine, 2011) and are considered as one of major causes of physical disability. The impact of wounds on physical, social and financial aspect of a person's life necessitate good wound management not only for the individual but for the community as well. For wounds to rapidly heal, complications from colonization of microorganisms must be avoided. The complications can be avoided by proper sanitation and good hygienic practices and proper medications (Lynch, 1989).

In rural communities, researchers observed that some therapies are usually initiated at health facilities for care and management of wound to promote healing and avoid infections and complications. In the health care centers, antiseptics and wound healing applications are done. Of the many antimicrobial agents available, iodophor-based formulations such as povidone iodine have remained popular of use. These are the most common antiseptics given for wound healing especially for fresh, uninfected wounds.

Povidone iodine remains to be popular and commonly used due to its favorable efficacy and tolerability. It has a broad spectrum of activity, ability to penetrate biofilms, lack of associated resistance, anti-inflammatory properties, low cytotoxicity, good tolerability and no negative effect on wound healing. These factors have been observed and cited as important in clinical practice. Over the past decades, numerous reports on the use of povidone iodine have been published, and more data supports its use on wound healing. However, there are cases and most often than not, patients prefer to simply stay at home and nurse themselves to care for their wounds. This is a common observation particularly when wounds are simply uninfected and do not require complicated and sophisticated treatments. Due to limited

resources to buy medicines and sometimes access to medical treatments, they choose to stay at home and resort to local herbs and plants for treatments (Novaes, *et al.*, 2016).

According to official fact sheets and reports (Kiran, *et al.*, 2015) and the press (BBC News 2014; Modern Ghana 2014), 80% of people in Asian and African countries (or sometimes that 80% of the world's population) use traditional medicine (TM) practices to meet their primary healthcare needs (Leoni, *et al.*, 2016). One of these herbal plants used for medicinal purposes is guava. Guava (*Psidium guajava* Linn) is one of those traditional medicinal plants used for treating wounds. Thriving in all types of soils, it is native and indigenous to the Philippines and can be found in almost all places in the country (Adao, 2016) with a long history of traditional use. A good proportion of which have been validated by scientific research (Kiran, *et al.*, 2015). Guava is also used medicinally in many parts of the world as an anti-inflammatory and antiseptic. The leaves are applied to wounds, ulcers and joints (for the relief of rheumatic pain) and are also chewed to relieve toothache (Cheng, 2011).

The wound healing effects of guava had been evaluated in several studies proving its effectiveness as supported by published reports. However, povidone iodine had been the most popularly used commercially-based antimicrobial formulation for wound healing. Moreover, the researchers had come up with this study as they found out that comparing these two treatments is interesting to investigate. The present study is aimed at evaluating the antimicrobial potential of leaf extracts of guava as compared to povidone iodine for wound healing in mice.

2. MATERIALS AND METHODS

All the materials needed for the study were brought into the facilities of the University of Eastern Philippines College of Veterinary Medicine (UEP-CVM). Commercially prepared povidone iodine (10% solution) and distilled water/plain NSS were procured from a drugstore. Processing of guava leaf extract and the induction and treatment of experimental wound incision to laboratory mice was done at the UEP-CVM laboratory facility. The same procedure and process of wound application of the treatments was followed for all animals under study.

3. METHODOLOGY

Collection and preparation of Guava Leaf extract

Young guava leaves were collected within the vicinity of the University of Eastern Philippines. In cases where there was shortage of supply of young guava leaves, it was sourced out from the neighboring areas within the municipality of Catarman. Soon after collection, the leaves were washed thoroughly in a running tap water and placed in a strainer to drain the water. Extraction was carried out using an electric juicer to obtain a liquid form of extract. The extract was being filtered using a mesh or muslin cloth. Extracted guava juice was being placed in a sterile container and stored in the body of the refrigerator in order to preserve the freshness and the active particles present in it.

Ethical Consideration

This study was carried out in strict accordance with the recommendations and approved Protocol in the Guide for the Care and Use of Laboratory Animals of the UEP-CVM's Institutional Care and Use Committee. All animals were treated under sedation with minimal pain upon incision.

Study Animals

BALB/c laboratory mice of either sex having a homogenous characteristic weighing approximately 20-30 grams from 4-6 weeks old were purchased from the University of the

Philippines College of Public Health Central Animal House and were used for the study. They were taken from UP Manila animal research laboratory, housed in cages individually and maintained under standard conditions at the University of Eastern Philippines College of Veterinary Medicine Laboratory Room. They were fed on standard pellet with water ad libitum maintained at 12 hours of light and 12 hours' dark cycle for 12 days in the departmental laboratory for acclimatization. All the mice were assessed for physiological parameters and only those apparently healthy were considered fit for surgical procedure, thus were considered in the study.

Grouping of Animals

Animals were divided into three (3) groups, consisting of six (6) mice each. Every group were housed individually in the laboratory cages. They were given free access to standard feeds and drinking water and were maintained on a 12-hour light/dark cycle.

Group I: This were treated with pure guava leaves extract (100%).

Group II: This were treated with standard povidone iodine (10% solution).

Group III: This were treated with distilled water and served as the placebo or control group.

Povidone Iodine Solution

Povidone iodine solution (10% solution) commercially prepared and obtained from a drugstore was used as one of the treatments in this study. A small amount of povidone more or less enough to cover the wounded area with the use of a dropper was the method used. A cotton swab was also used to cover the wounds at approximately 2cm around the surroundings of the wound following manufacturer's recommendation. After applying the treatment, wounded area were left uncovered since it is difficult to cover it with sterile bandage. There is always the tendency that the mice will remove the dressing. Treatment was given once a day in the morning.

Plain NSS Solution

A plain NSS or distilled water was purchased from a drugstore and was used as treatment for the placebo or control group.

Experimental Design

This study was laid out in a single factor completely randomized design (CRD) with balanced replication. A total of eighteen (18) BALB/c mice were randomly divided into three (3) treatment groups with three (3) animals in each group. Each animal served as a replicate. Replicate Treatment zero (RT0) served as the untreated/negative control group given with distilled water. RT1 and RT2 were the positive control groups. RT1 was treated with 100 percent guava leaf extract, and RT2 was treated with povidone iodine (Betadine at 10% solution).

Anesthesia and Wound Creation

Surgical procedure was aseptically performed in the animal facility operating room of the College of Veterinary Medicine. Eighteen (18) mice were divided into three (3) groups with six (6) laboratory animals in each. All animals in each group was anesthetized by intraperitoneal (IP) injection of Zoletil 50 at .01 ml dosage before the incision. A 2 cm full-thickness skin wound was incised from the dorsum of the laboratory mice. Incision was made through the skin and cutaneous muscle at a distance of 2 cm from the midline on one side of the depilated back of the mice aseptically. A single incision was made to each of the eighteen (18) mice. Using aseptic surgical technique, a 2 cm length and in full-thickness incision was made using a number 10 scalpel blade. On the other hand, a ruler (caliper) was used as a

guide on the incision length. Hemorrhage of wound was controlled by compression using sterile gauze. The wound incision was left unsutured for secondary intention healing for 24 hours. The day of wound creation marked the day 0 of the experiment. Application of the formulated test material commenced after 24 hours post-wounding. (Sabale, *et al.*, 2012).

The first group received pure guava leaf extract (100%), the second group with povidone iodine (Betadine at 10% solution), and the third group with placebo (distilled water). The mice were observed daily. The treated area was measured and observed for evidence of healing.

Histologic Grading of Wound Healing

A 0.5 cm biopsy specimen was taken from the wound edges of each representative mouse on its designated groups on days after 9 days. The specimen was fixed in a 10% buffered formalin solution and samples were sent for processing to a private pathology laboratory at University of the Philippines, Los Baños, Laguna. The section of the skin samples will be stained with eosin and hematoxylin and examined microscopically under low power to high power objective.

Levels of collagen tissue, angiogenesis, fibrosis and epithelialization will be evaluated using the Abramov's histological scoring system which is a modified Greenhalgh's scoring system. A score of 0-3 will be used on each parameter independently. The collagen level will be graded as: 0 (none), 1 (scant), 2 (moderate), and 3 (abundant). Angiogenesis will be graded as either: 0 (none), 1 (up to 5 vessels per high power field [HPF]), 2 (6–10 vessels per HPF), and 3 (more than 10 vessels per HPF). Fibrosis will be graded as: 0 (none to minimal fibroblasts), 1 (few fibroblasts), 2 (more fibroblast) and, 3 (predominantly fibroblasts). While epithelisation will be graded as either: 0 (none), 1 (partial), 2 (complete, but immature or thin), and 3 (complete and mature) to compare the following treatments:

Data Analysis

Evaluation of the macroscopic and microscopic changes of the wound incision was based on the test of normality, significance of the treatment in which covariate will be checked for external factor affecting the wound healing in mice.

4. RESULTS AND DISCUSSION

The results of the study which determined the macroscopic wound healing efficacy of guava leaf extracts and povidone iodine on the incised skin of laboratory mice in terms of time by describing its characteristics is presented in in terms of:

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graded as either: 0 (none), 1 (partial), 2 (complete, but immature or thin), and 3 (complete and mature).

Data on histologic examination processed at UP Los Baños and analyzed based on the result of the examination as presented below:

CASE NUMBER: 19 – H136

Species: Mice

FINDINGS:LEGEND: 1 – None 2 – Slight/few 3 – Moderate 4 - Abundant

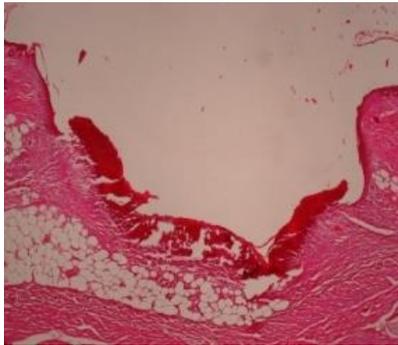
SLIDE NO.	MICE NO.	INFLAM CELLS	RBC	NEOVASCULARIZATION	EPITHELIAL REGENERATION	FIBROBLASTIC PROLIFERATION
1	25	4	4	2	1	1
2	12	4	3	2	2	2
3	19	1	2	3	4	4
4	21	1	4	4	1	3
5	5	1	2	3	1	2
6	17	1	4	3	1	3
7	9	1	1	3	3	4
8	13	1	4	3	2	3
9	20	1	4	2	1	2
10	3	1	4	1	1	1

NOTES:

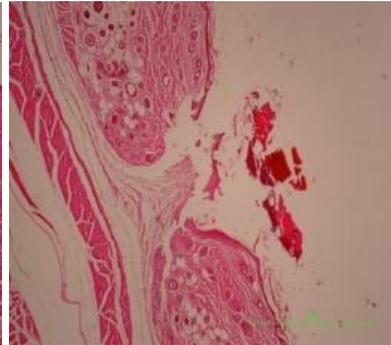
SLIDE NO.	MICE NO.	Description
1	25	There is no epithelial layer present; hemorrhage exudation, moderate neovascularization with slight fibroplasia; scab formation with inflammatory cells present
2	12	Moderate RBCs with slight neovascularization and fibroplasia; Epithelial apposition noted; scab formation with inflammatory cells
3	19	More of hemorrhagic with many RBCs without inflammatory cells beneath the layer (Looks like a closed lesion in one slide made)
4	21	There is much scab formation; many RBCs in the epithelium and neovascularization prominent
5	5	There is much scab formation; slight fibroplasia; moderate neovascularization and slight RBCs present
6	17	Numerous RBCs; No inflammatory cells present; Epithelial regeneration is slight or starting with moderate fibroplasia
7	9	There is moderate neovascularization and abundant fibroblastic proliferation with moderate epithelial regeneration; scab formation
8	13	RBCs are numerous; Abundant neovascularization; moderate fibroblast proliferation; and slight epithelial regeneration; scab formation
9	20	Almost same as number 8 – Mice no. 13
10	3	Many RBCs present with abundant fibroblastic proliferation; moderate epithelial regeneration; inflammatory cells not present

• RBCs present means outside the blood vessels/capillaries

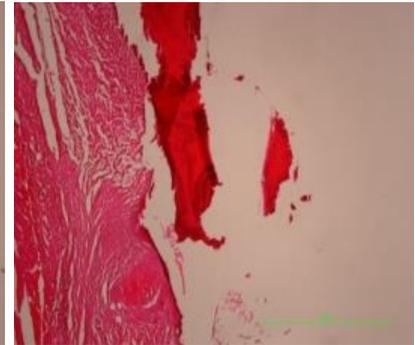
Scale: 4X = 1um 10X =23um
VERONICA A. MATAWARAN Veterinary Pathologist



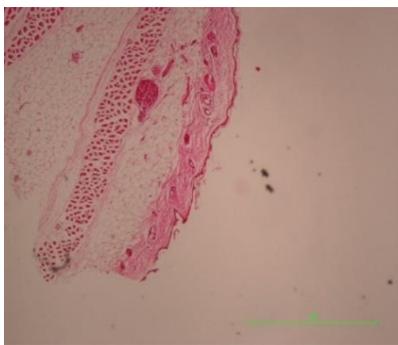
Mice No. 25



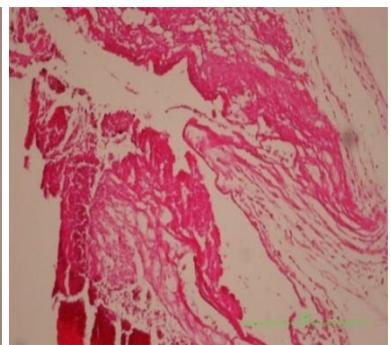
Mice No. 12



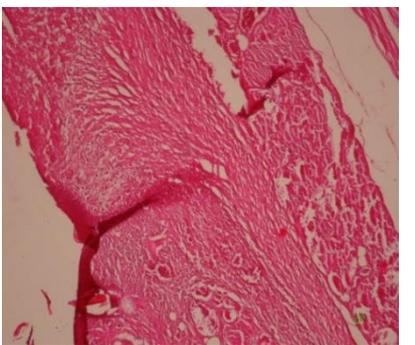
Mice No. 21



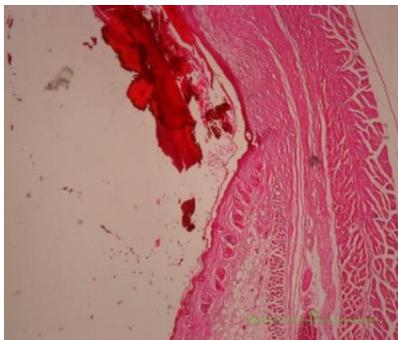
Mice No. 19



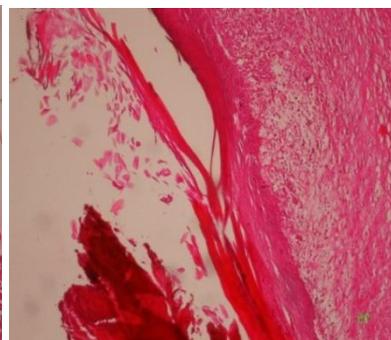
Mice No. 5



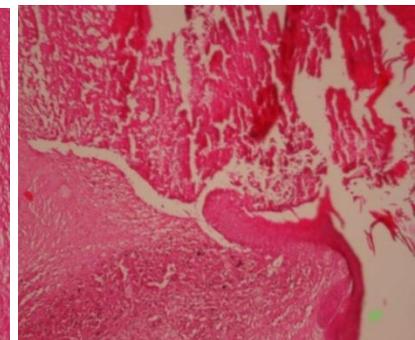
Mice No. 17



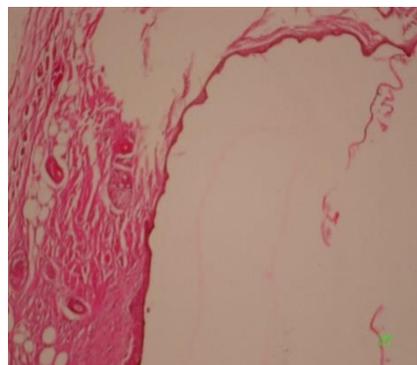
Mice No. 9



Mice No. 13



Mice No. 20



Mice No. 3

The data revealed that laboratory mice treated with pure GLE (100%) has manifested moderate swelling from Day 1 to Day 2 and a slight negative swelling from Day 3 to Day 9. Mice treated with PI solution had moderate swelling on the 1st day observation but some exhibited prominent swelling on the succeeding days (5th day) and moderate swelling until the 7th day and became slightly negative on the 7th and 9th day.

Those treated with PNSS all mice had moderate swelling of wounds on the 1st until 3rd day of observation and one become prominent on the succeeding days (7th and 8th day) until it became slightly negative on the 9th day. For wound elevation, group 1 treated with GLE had on the 3rd day of observation, three (3) mice had the absence of elevated wounds while three (3) with slight wound elevations that disappeared on the 5th and 6th day of observation. For Group II with PI (10% solution) treatment, (2) mice were observed to have absence of wound elevations, one (1) had moderate elevation and three (3) manifested slight wound elevations. With PNSS treatment, two (2) exhibited moderate wound elevations while four (4) were with slightly elevated wounds. In observing for hyperemia or excessive bleeding, the result showed absence of hyperemia or excessive bleeding for all the three groups of laboratory mice given three different wound treatments (pure GLE (100%), PI 10% solution, and PNSS) under study.

The result revealed that all mice which received guava leaf extract formed scar earlier as compared to povidone iodine and PNSS. Since maturation phase which also refers to remodeling phase is responsible for the new epithelium and final scar tissue formation, and as the development of these completes the complex process of wound healing, the researchers suggest that the use of guava leaf extract in comparison with povidone iodine and PNSS when it comes to wound healing is the most effective treatment in promoting normal and more rapid wound healing.

5. REFERENCES

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