

EPIDERMAL WOUND HEALING ACTIVITY OF ALOE VERA LEAF AQUEOUS EXTRACT

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ABSTRACT

This study aimed to determine the wound healing activity of Aloe vera (Aloe vera) in female Sprague Dawley rats. Aqueous Aloe vera extracts (Aloe vera) were prepared by grinding. Thirty-five female Sprague Dawley rats weighing 150-250grams were randomly divided into six groups: group I (n=5) Positive control rats; group II (n=5) rats treated topically with 25% Aloe vera aqueous leaf extract; group III (n=5) rats treated with 50% Aloe vera aqueous leaf extract; group IV (n=5) rats treated with 75% aloe vera aqueous leaf extract; group V (n=5) rats treated with 100% aloe vera aqueous leaf extract; group VI (n=5) as the Negative control. The wound closure was observed and measured on the days 0, 2, 4, 6, 8 and 10. Furthermore, Statistical analysis using Student T-test and One-way Analysis of Variance (ANOVA) showed that the 75% aqueous Aloe vera extract is the same with the positive control and 100% aqueous Aloe vera extract is comparable with the positive control. The excised skins from all the treatments were processed for histopathological examination. Based on the results, it was found that the 75% and 100% aqueous extract of Aloe vera (Aloe vera) are comparable to the wound healing activity of Mupirocin, a potent wound healing agent. Thus, it can be concluded that the aqueous extract of Aloe vera (Aloe vera) possesses potential wound healing activity which can be used as an alternative wound healing agent which are normally costly.

Keywords: *Aloe vera, wound healing activity, aqueous extract, epidermal wounds, Histopathological examination*

INTRODUCTION

Wounds are serious public health problem regardless of gender, age and race. Successful wound healing process requires deeper understanding on the wound healing process and the factors that can disrupt wound healing or wound repair (Rebolla et al., 2013). Wound healing is a systemic process, which is marked by an injury and continues with a series of physiologic responses that will ultimately impact the ability of the wound to heal. Factors including age of patient, type of wound, patient behavior, environment, type of wound care the patient is receiving and chronic diseases. Thus, optimal rate of healing is attained when factors advantageous to healing are present and factors having the ability to interfere with healing process are controlled or inhibited (Philips, 2000).

Wound healing caught the medical world's attention and became a major interest because skin wounds affect a large quantity of patients, significantly reducing their quality of life, and increase on their health care expenditures due to longer hospitalization time (Perini et al, 2015).

Generally, wounds can be classified into three categories; Superficial which is loss of epidermis, partial thickness which involves the loss of dermis and epidermis and full thickness which involves the dermis, subcutaneous fat and sometimes bone (Flannagan, 1997).

Normally, when people get wounded, they immediately wash the wounded area with clean water, antiseptics or anti-infective, application of ointments or creams and dressing, and the use of alternative medicines such as herbal plants.

Aloe vera (*Aloe vera*) also known as the "healing plant" has gained a lot of attention in the medicine world due to its various pharmaceutical and medicinal properties. *Aloe vera* is a cactus-like plant which belongs to the family of *Liliaceae*, that grows on hot and dry places (Vogler and Ernst, 1999) Aloe vera has various medicinal uses like anti-inflammatory, treatment for ulcers and hepatitis. It is also said to be beneficial in treating acne (Reynolds and Dweck, 1999) and also for treating diabetes (Noor et al., 2008)

The study was conducted to alleviate the problems and complications of wounds. Thus, this study aimed to provide scientific evidence in the wound healing effect of aqueous extract of Aloe vera (*Aloe vera*) particularly the leaves.

Research Questions

Generally, this study aimed to evaluate the wound healing activity of Aloe Vera extract in Sprague Dawley Rats and to answer the following questions:

1. What are the phytochemical constituents present in the Aloe Vera (*Aloe vera*) leaf extract that promotes wound healing?
2. What is the epidermal wound healing activity of the different treatments in terms of:
 - a. Measurement of wound
 - b. Percentage closure of wound area
3. What are the histopathological characteristics of the wounds of the test subjects after administration of different treatments?
4. Is there a significant difference on the healing activity of the different aqueous extract concentrations?
 - a. 25% aqueous aloe vera extract
 - b. 50% aqueous aloe vera extract
 - c. 75% aqueous aloe vera extract

- d. 100% aqueous aloe vera extract
5. Is there a significant difference in the wound healing activity on the different aqueous extract concentrations?
 1. Positive control (Mupirocin 2%) vs. 25%, 50%, 75% and 100% Aloe vera aqueous extracts.
 2. Negative control vs. 25%, 50%, 75% and 100% Aloe vera aqueous extracts.
 3. Positive control (Mupirocin 2%) vs. Negative control

Hypotheses

HO1. There is no significant difference on the wound healing activity of the 25%, 50%, 75% and 100% Aloe vera aqueous extract concentrations.

HO2. There is no significant difference on the wound healing activity of the 25%, 50%, 75% and 100% Aloe vera aqueous extract concentrations and Positive control

HO3. There is no significant difference on the wound healing activity of the 25%, 50%, 75% and 100% Aloe vera aqueous extract concentrations and Negative control

HO4. There is no significant difference on the wound healing activity of Positive control (Mupirocin 2%) and Negative control

Significance of the Study

This study provides significance in decreasing the complications of wound healing. To help the public by providing a safe, effective and low cost wound healing agent, promote safe and efficacious use of folkloric medicinal plant in the modern time and to discover and develop organic based hastened healing process of Aloe vera (*Aloe vera*) in superficial wounds.

Literature Review

Wounds According to Depth

Wounds can be identified by several methods which include their etiology, location, type of injury or manifestation of symptoms, wound depth and tissue loss and even the clinical appearance of the wound. Additionally, there is a separate criterion for the categorization of wounds according to depth which is classified as a) superficial wounds which is manifested by the loss of epidermis only; b) partial thickness which involves the epidermis and dermis; and lastly c) full thickness which involves the dermis, subcutaneous fat and sometimes bone (Flannagan, 1994).

The Stages of Wound Healing

Wound healing is a complex and dynamic process of replacing devitalized and missing cellular structure and tissue layers. (Mercandetti M. et al., 2017). The wound healing process in any type of wound is categorized into three phases; inflammatory phase, proliferate phase and the remodelling phase. (Nayak S. et al., 2017). Within these three phases are several sub processes which include chemotaxis, phagocytosis, neocollagenesis, collagen degradation and collagen remodelling, angiogenesis and epithelisation. The peak of these processes results in the production of new tissue. (Simon P., et al, 2018).

Management of Superficial Wounds

Superficial wounds are wounds that involve the epidermal layer of the skin only even the slightest breakage or injury to the skin which acts as a barrier that prevents the entry of pathogens and foreign particles from invading the body. (A. Khan, Kotta S. et al., 2013). Thus, wound care and management is essential to prevent further infections and more serious complications. For the most part, typical first aid measures are applied to counteract the first symptoms of wounds; these includes controlling the bleeding, washing the wound, applying topical antimicrobial ointment or wound healing ointment in the affected area. (Marusinec, L., et al, 2015). Several traditional and herbal wound care products are also currently used in the management of wounds; these herbal agents include honey from (*Apis mellifera*). (Ananda Dorai., 2012). The essence of papaya (*Carica papaya*), horse radish (*Moringa oleifera*), guava (*Psidium guajava*) and Lagundi (*Vitex negundo*) are also used as alternative and traditional agents for the care of wounds. (Patel DK., 2014).

Traditional and Alternative Medicine Act (TAMA) of 1997

Medicinal plants have been an inspiration for the creation of numerous therapeutic drugs, as plant derived drugs have made contribution to the health and wellness of humans. According to the World Health Organization (WHO), approximately 85% of the global population depends on herbal plants for their primary health care.

As more people recourse to alternative medications, the emphasis on the promoting the use of herbal plants in the country should be set in motion to attain affordable healthcare system. As an important and natural source of nutrition and substances which produce physiological action on the human body (Olowa and Nuñez, 2013),

The Republic Act No. 8423 also known as Traditional and Alternative Medicine Act (TAMA) of 1997 strives for the development and application of traditional and alternative medicine in the national health care delivery system. This act also

promotes the importance of traditional medicine in the country as it has been embedded in our culture since the pre-Hispanic era.

This act emphasizes scientific research, promotion, and advocacy on the use of traditional, alternative, preventive and curative health care modalities and formulation of standards, guidelines, and codes of ethical practice appropriate for the practice of traditional and alternative health care.

Aloe vera (*Aloe vera*) Ethnopharmacology

Aloe vera Linn. (Liliaceae) syn. *Aloe vera* Miller, which is also known as the "Healing Plant" is a cactus-like plant that grows in hot, dry places such as Africa and India. There are almost 360 species of *Aloe* grown in dry regions around the world. It is most commonly used as an anti-inflammatory agent, and anti-fungal agent. (Oryan A. et al., 2010).

Aloe vera is the most commercialized *aloe* species because of its wide uses such as in cosmetics, toiletry, food and pharmaceutical industry. (F. Nejatizadeh-Barandozi, 2013) *Aloe* has been marketed as treatment for cough, gastritis, headaches and it is also used as laxative. (Rajeswari, R., Umadevi, M., Rahale et al., 2012) It has been used as a medicinal agent in different cultures for almost a thousand years (A. Khan, Kotta S. et al., 2013) and was said to treat several disorders such as gout, acne, skin diseases and can be used as an anti-infective. (Oryan A. et al., 2010).

Aloe vera contains at least 6 antiseptic agents such as lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols and sulphur. These 6 constituents can kill or control molds, bacteria, fungi and viruses, which explains why the plants has been claimed to be effective in eliminating internal and external infections. (Rajeswari, R., Umadevi, M., Rahale et al., 2012)

Pharmacologically, it boosts the immunity and detoxifies the system. It is recommended as an adjuvant therapy for antibiotics, NSAIDs (Non- Steroidal Anti Inflammatory Drugs) and chemotherapy to eliminate drug induced gastritis. It is also said to be useful in several types of diseases such as Diabetes mellitus II, asthma, bronchitis, eye diseases, tumor, spleen enlargement, liver complaints and it can also maintain a good gastric pH and relieve constipation. (Rajeswari, R., Umadevi, M., Rahale et al., 2012)

A recent report showed that *Aloe vera* possesses constituents which can improve the wound healing process, activates macrophages, demonstrate antineoplastic and antiviral activities. It has also been shown to be a possible therapeutic agent for sepsis and hepatotoxicity (V. Saritha et al., 2010).

Research Paradigm

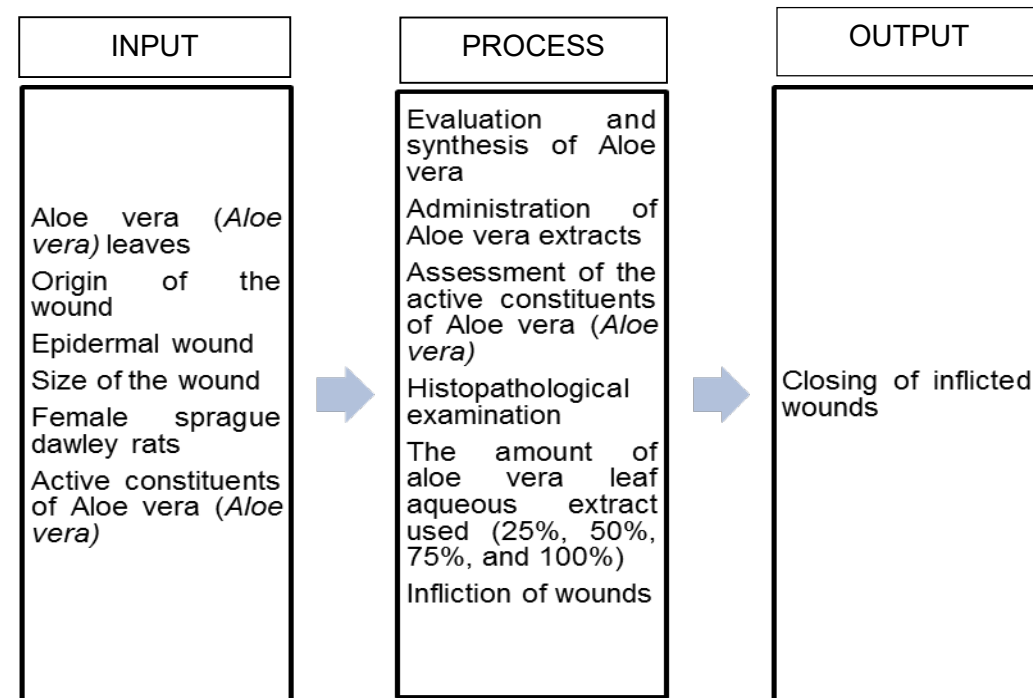


Figure 1. *Research paradigm*

Figure 1 shows the variables that were used in this study. *Aloe vera* (*Aloe vera*) was subjected into evaluation. The wound healing activity of *Aloe vera* was tested through conduct of variety of tests and will be compared to the control. The results of this study were able to show the wound healing activity of *Aloe vera* (*Aloe vera*).

METHODS

This study includes different equipment, methods, and procedures for the activity of the leaves of *Aloe Vera* for wound healing. The research methodology, subject of the study, sampling technique, procedure of gathering and statistical treatments are shown below:

Research Design

Experimental method was used in this study. It was done by treating rats with excised wounds with the Aloe vera leaf extract and Purified water which was used as the negative control. Data were statistically analysed using +S.E.M and $p < 0.05$ was considered being the criterion for statistical significance (M. Abdullah et al. 2010). The wound healing activity of the extract was introduced to 6 groups, with 5 rats per group.

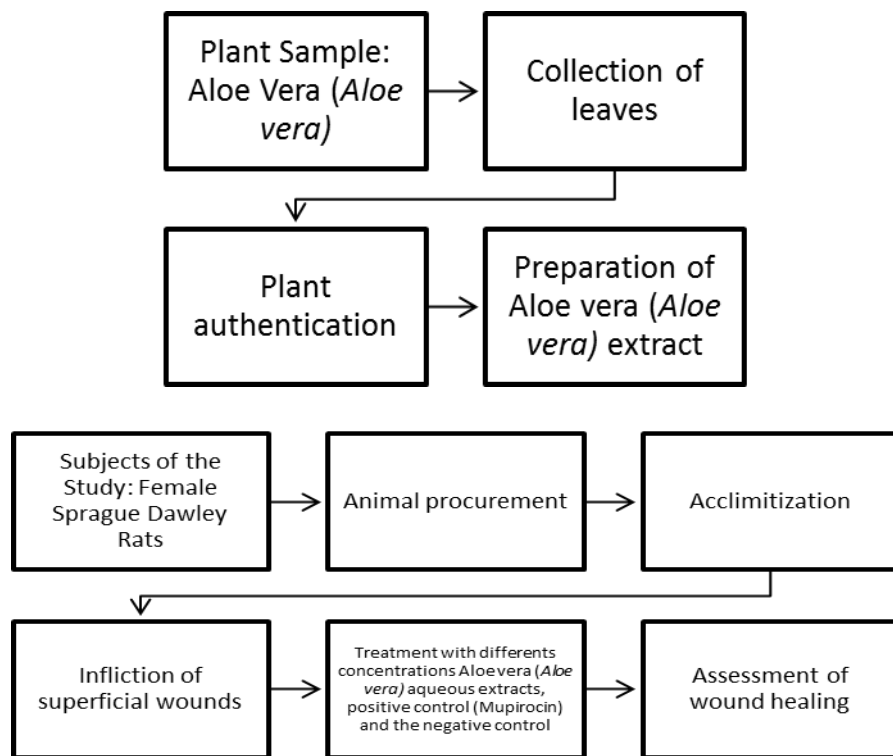


Figure 2. Methodological flow chart

Figure 2 shows the general methods that the researchers used in the study, from the collection of the leaves of Aloe vera (*Aloe vera*) to the extraction of the leaves and the testing for its wound healing activity.

Data Gathering Procedures

This includes various processes this research study underwent namely plant collection, phytochemical screening, histopathological examination of wound healing, data analysis, ethical considerations and waste disposal management.

1. Plant Collection and Preparation of the Plant Sample and Extraction of Aloe vera (*Aloe vera*) aqueous extract

- 1.1 Thick succulent leaves of Aloe vera (*Aloe vera*) plant obtained from different parts of Tuguegarao City were used and authenticated at the Department of Agriculture in Tuguegarao City.
- 1.2 To make the gel thicker, the plant's leaves were given sufficient time to be relatively dried at room temperature without exposure to direct sunshine.
- 1.3 After adding a small quantity of water, the leaves were ground in a blender and centrifuged at 10,000 g,
- 1.4 The fibers were removed and then filtered through filter papers the stored at +4 °C controlled room temperature.

2. Phytochemical Screening

250ml of Aloe vera (*Aloe vera*) aqueous leaf extract was sent to the phytochemical screening laboratory of Saint Louis Baguio to undergo different identification tests to detect the presence of metabolites.

3. Preparation of the Test Subject

- 3.1. Thirty-five female Sprague Dawley rats weighing 150-250grams were obtained and housed under standard conditions of temperature, 12 hour light/dark cycle and fed with standard pellet diet and water *ad libitum*.
- 3.2. The experimental rats were kept in polypropylene cages with paddy husk bedding and were acclimatized for one month under the aforesaid housing, feeding and other provided conditions.

4. Infliction of superficial wound

- 4.1. The animals were anaesthetized using Zoletil 50.
- 4.2. The anesthetized animals were secured to the operation table in the natural position.
- 4.3. The fur of the dorsum of each animal was shaved and wound area was created.

- 4.4. Povidone Iodine was applied as an antiseptic for the shaved region before the wound creation.
- 4.5. An impression was made on the dorsal interscapular region 5 mm away from the ears using a circular collared rubber stamp of 21 mm diameters as described by Morton and Malone.
- 4.6. The epidermis from the demarcated area was excised.
- 4.7. The wound was blotted with a cotton swab soaked in normal saline.
- 4.8. The treatment included topical application of 25%, 50%, 75% and 100% aqueous Aloe vera gel extract compared with a positive group treated with Mupirocin (Foskina) and a negative control group. Treatments were continued until complete closure of the wound occurred.

5. Experimental Protocol

After wound creation, experimental animals were randomly divided into six groups: group I (n=5) Positive control rats; group II (n=5) rats treated topically with 25% Aloe vera aqueous leaf extract; group III (n=5) rats treated with 50% Aloe vera aqueous leaf extract; group IV (n=5) rats treated with 75% aloe vera aqueous leaf extract; group V (n=5) rats treated with 100% aloe vera aqueous leaf extract; group VI (n=5) as the Negative control. Three animals in each group were used for studying progressive changes in morphology of wound. One animal in each group was used for histopathological and biochemical analysis.

6. Assessment of wound healing

The physical attributes of wound healing namely, wound closure, epithelialisation time and scar features were studied by tracing the raw wound area on tracing paper on the days 0, 2, 4, 6, 8, 10, 12, 14 and 16. Wound area was calculated by the number of squares of the retraced wound area on a 1mm² graph paper from the tracing paper. The degree of wound healing was calculated as percentage closure of the wound area from the original wound area using the Walker and Mason formula:

$$\text{Percentage closure} = 1 - \frac{A_d}{A_0} \times 100$$

where A_0 is the wound area on the day zero and A_d is the wound area on corresponding days.

7. Histopathological Examination of Wound Healing

- 7.1. Wound tissue specimens from control, test and standard groups were taken after complete healing of excised wound and after usual processing 6-mm thick sections was cut and stained with hematoxylin and eosin.

- 7.2. Sections were qualitatively assessed under the light microscope and observed in respect of fibroblast proliferation, collagen formation, angiogenesis and epithelialization.

Data Analysis

The data gathered were tabulated and subjected to statistical treatment, utilizing the One-way Analysis of Variance (ANOVA) using 0.05 level of significance and Least Significant Difference (LSD) for comparative analysis

Ethical Consideration

This research study underwent the University Research Ethical Board (UREB)'s review of protocols prior to its conduction of experiment. This research was approved by the academic dean, associate dean, and the vice president for academics of this university. The researchers guarantee that safety laboratory protocols were strictly followed during and after the conduct of the study.

After the experimentations, all animal models were euthanized using the Carbon dioxide mass suffocation technique. After ensuring that all rats were euthanized, the carcasses were disposed using the Pyre Burning Technique, the carcasses were incinerated in an open system of fire which was fuelled by additional materials of high energy content in an open field. After incinerating the carcasses, the ashes were buried 3 feet underground to prevent the wind to scatter it.

RESULTS

Table 1. *Phytochemical Screening of the aqueous extract of Aloe vera leaves*

Constituent	Result
Alkaloids	(+)
Carbohydrates	(+)
Glycosides	(+)
Saponins	(-)
Phytosterol	(-)
Phenolic Compounds	(+)
Flavonoids	(+)
Protenins	(-)

Phytochemical screening of the aqueous leaf extract of Aloe vera (*Aloe vera*) is positive for the presence of alkaloids, carbohydrates, glycosides, phenolic compounds and flavonoids.

Table 2.1. Measurement of *Wound Closure before and after Administration of Different Treatments*

Treatment	After Infliction of wound	After extract administration				
		Day 2	Day 4	Day 6	Day 8	Day 10
Treatment 1 (25% Aloe vera extract)	5.60	5.20	3.40	2.10	1.70	.60
Treatment 2 (50% Aloe vera extract)	6.00	5.40	3.80	1.88	1.80	.75
Treatment 3 (75% Aloe vera extract)	4.80	4.00	3.20	1.60	1.50	.25
Treatment 4 (100% Aloe vera extract)	4.80	4.40	2.40	1.20	1.10	.40
Positive control (Mupirocin)	4.40	4.00	2.60	1.34	1.24	.25
Negative Control (Normal Saline)	3.40	4.00	3.00	3.60	3.60	2.00

This table reveals that the positive and experimental groups which are 75% and 100% have comparable mean number of wound healing effect during the 6th, 8th, 10th day. Almost all the wounds completely closed at the 10th day. The experimental groups 75% is the same with positive control and 100% is somehow close to the positive control.

Meanwhile, the negative group and experimental groups 25% and 50% respectively have slow onset of wound healing activity on the rats.

Table 2.1. *Percentage Closure of Wounds after Administration of Different Treatments*

Treatments	Day 2	Day 4	Day 6	Day 8	Day 10
Treatment 1	7.14	39.29	62.50	69.64	89.29
Treatment 2	10.00	36.67	68.67	70.00	87.50
Treatment 3	16.67	33.33	66.67	68.75	94.79
Treatment 4	8.33	50.00	75.00	77.08	91.67
Positive control	9.09	40.91	69.55	71.82	94.32
Negative Control	-17.65	11.76	-5.88	-5.88	41.18

Table 3. *Histopathological Evaluation of the Wounds of Test Subjects after administration of Different Treatments*

Treatments	Histopathologic Description
Treatment 1	Shows no signs of wound healing
Treatment 2	Shows a very minimal sign of wound healing
Treatment 3	Manifests signs of wound healing such as neovascularization and deposition of extra-cellular wound matrix.
Treatment 4	Shows commendable amount of neovascularization, deposition of extra cellular matrix and development of skin appendages which are manifestations of wound healing.
Positive control	Shows commendable amount of neovascularization, deposition of extra cellular matrix and development of skin appendages which are manifestations of wound healing.
Negative Control	no signs of wound healing

The table shows the results of the histopathologic evaluation of the wound specimens from the subjects belonging to different treatment groups. The table further shows that the specimen of subjects that received Treatment four has the same histopathologic characteristics as those which received the positive control. This therefore signifies that 100% Aloe vera extract has the same effect as the positive control (Mupirocin).

Table 4.1. Test of Significant Difference in the Measurement of Wound Closure of the Different Treatments grouped according to Number of Days Post-treatment

	F-value	p-value	Decision
Day 2	2.874	.036	Reject Ho
Day 4	2.857	.037	Reject Ho
Day 6	4.754	.004	Reject Ho
Day 8	5.333	.002	Reject Ho
Day 10	7.642	.000	Reject Ho

The table above shows that there is a significant difference in the measurement of wound closure according to the number of days of administration of the different treatments.

Table 4.2. Multiple Comparisons of the Significant Difference in Measurement of Wound Closure 6 Days Post-Treatment

Treatments	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Positive control	Negative Control
Treatment 1	1					
Treatment 2	.701	1				
Treatment 3	.386	.625	1			
Treatment 4	.125	.241	.487	1		
Positive control	.192	.350	.650	.807	1	
Negative Control	.014*	.006*	.000*	.000*	.001*	1

*Significant at 0.05 level

The table above shows that all the concentrations of Aloe vera extract have significantly similar wound healing effect as the positive control (Mupirocin). Furthermore, No significant difference in the measurement of wound closure was noted among the different concentrations of Alo vera extract.

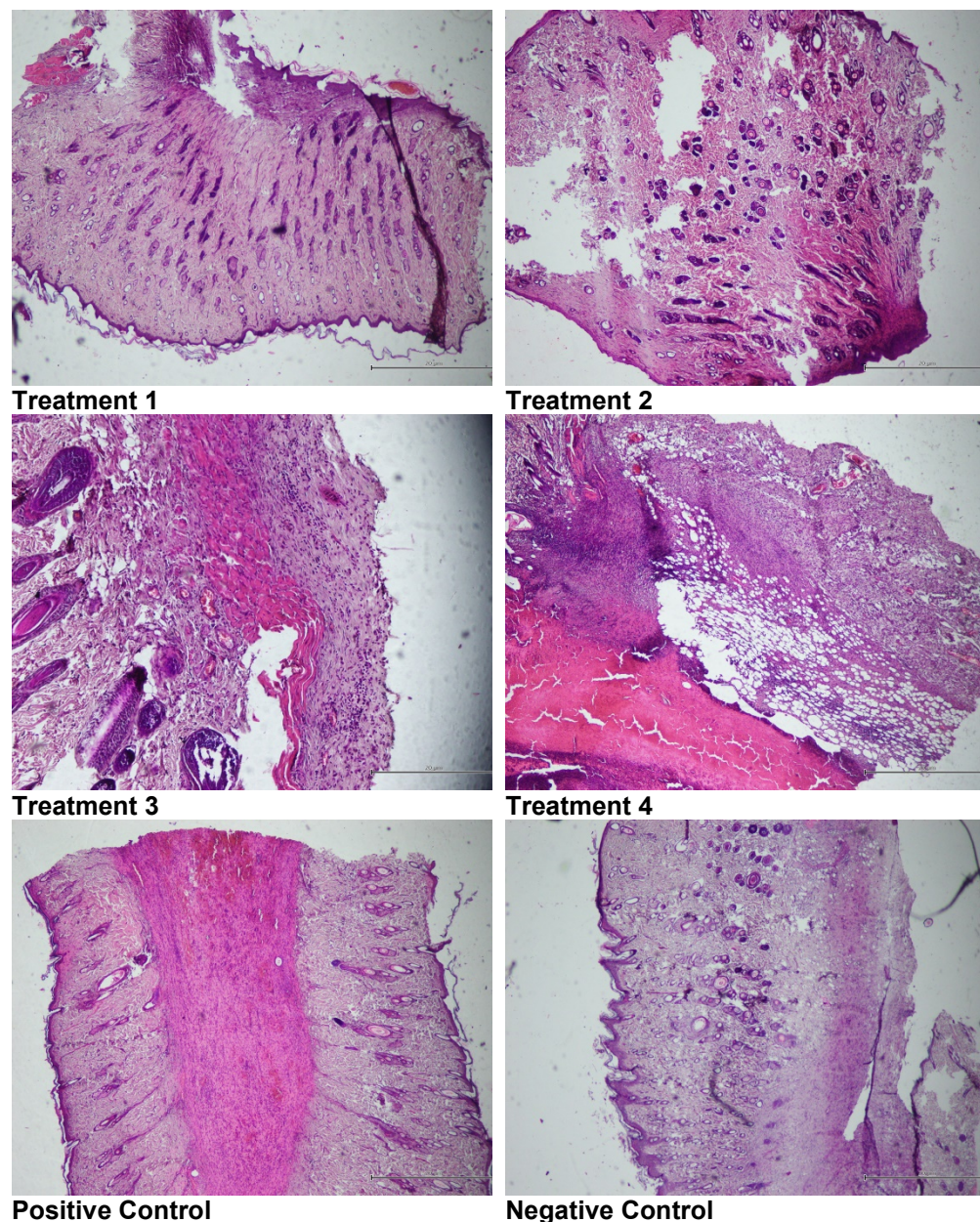


Figure 3. Microscopic View of the Histopathological Characteristics of the Wounds of Test Subjects after Administration of Different Treatments

Table 4.3. Multiple Comparisons of the Significant Difference in Measurement of Wound Closure 8 Days Post-Treatment

Treatments	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Positive control	Negative Control
Treatment 1	1					
Treatment 2	.701	1				
Treatment 3	.386	.625	1			
Treatment 4	.125	.241	.487	1		
Positive control	.192	.350	.650	.807	1	
Negative Control	.014	.006	.000	.000	.001	1

*Significant at 0.05 level

The table above shows that all the concentrations of Aloe vera extract have significantly similar wound healing effect as the positive control (Mupirocin). Furthermore, No significant difference in the measurement of wound closure was noted among the different concentrations of Alo vera extract.

Table 4.4. Multiple Comparisons of the Significant Difference in Measurement of Wound Closure 10 Days Post-Treatment

Treatments	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Positive control	Negative Control
Treatment 1	1					
Treatment 2	.701	1				
Treatment 3	.386	.625	1			
Treatment 4	.125	.241	.487	1		
Positive control	.192	.350	.650	.807	1	
Negative Control	.000*	.000*	.000*	.000*	.000*	1

*Significant at 0.05 level

The table above shows that all the concentrations of Aloe vera extract have significantly similar wound healing effect as the positive control (Mupirocin). Furthermore, no significant difference in the measurement of wound closure was noted among the different concentrations of Alo vera extract.

DISCUSSION

The study was intended to determine whether Aloe vera (Aloe vera) leaf aqueous extract has a wound healing activity which will, therefore, provide an alternative for wound healing preparations that may be used by patients who are suffering from wound-related conditions. Topical application of the extracts were done to allow high percentage of healing while avoiding systemic toxicity and are most effective in the earlier stages of wound healing prior to the formation of a solid granulation bed (Kanne, et al, 2012). In the study, phytochemical screening of constituents results show that Aloe vera leaves (Aloe vera) extract is positive for the following constituents: alkaloids, carbohydrates, glycosides, phenolic compounds and flavonoids while negative for saponins, phytosterol and proteins. This has also been the result of Vasani, et,al, 2008 in their short review about the plant, that Aloe vera (Aloe vera) contains 74 potentially active constituents namely: phenolic compounds, carbohydrates and glycosides. It was stated in their study that the plant possesses the polysaccharide glucomannan which is said to be helpful in the acceleration of wound healing. Glucommmanan in Aloe vera might be present because the Aloe vera extract was said to be positive in carbohydrates.

The Aloe vera extract in this research was applied topically, this was what Kanne, Gujjila et al, 2012 justified in their study that topical application of the extracts were done to allow high percentage of healing while avoiding systemic toxicity and are most effective in the earlier stages of wound healing prior to the formation of a solid granulation bed. For the determination of its wound healing activity, an animal model was used and Sprague Dawley rats weighing 200 to 250 grams were selected. Mupirocin was used as a reference drug for comparing the wound healing activity of the aqueous leaf extract of Aloe vera (Aloe vera). Four concentrations of Aloe vera extract such as 25%, 50%, 75% and 100% were used to study the wound healing activities of the said plant subject. The four concentrations of the aqueous extract manifested wound healing activity based on the wound contraction measurements that were recorded every other day.

According to Velnar et al, (2009), wounds are said to be in the proliferative phase of wound healing by the manifestation of fibroblast mitigation, deposition of newly synthesized extracellular matrix, abundant formation of granulation tissue, deposition of collagen material and neovascularization. Skin wound healing is a dynamic response to injury that results in wound contraction, wound closure and restoration of the functional barrier (Rajan et al., 2014). Al-Henhena et al. (2011) reported in their article that wound healing is a complex and dynamic process of

restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. According to Midwood, Williams, and Schwarzbauer (2004), wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. He also noticed that in the maturational phase, the final phase of wound healing, the wound undergoes contraction resulting in a smaller amount of apparent scar tissue. In connection to this, the wound healing activity of Aloe vera (*Aloe vera*) leaf aqueous extract was manifested basing it on the results of the statistical analysis of the wound contraction data from day 6, 8 and 10. Muhammad and Muhammad in their study noticed that the wound healing effects may be due to regulation of collagen expression and increase in tensile strength of the wounds. They also reported that enhanced healing activity has been attributed to increased collagen formation and neovascularization. (Al-Henhena N, et al 2011) based on their study reported that collagen plays a central role in the healing of wounds and it is a principal component of connective tissue and provides a structural framework for the regenerating tissue. A wound healing mechanism termed as angiogenesis or neovascularization improves the circulation to the wound site facilitating the oxygen and nutrients required for the wound healing process which involves re-epithelization. Aloe vera (*Aloe vera*) leaf aqueous extract specifically the 75% and 100% concentration have demonstrated large amount of extra cellular deposition, formation of granulation tissue, augmentation of collagenous material and commendable amount of neovascularization.

The histopathological studies revealed that animals treated with 75% and 100% Aloe vera (*Aloe vera*) leaf aqueous extract exhibited marked dryness of wound margins with tissue regeneration after 10 days of the experimental period. The histopathological evaluation showed that increased cellular infiltration from haematoxylin and eosin staining in treated cases may be due to chemo tactic effect enhanced by the crude extract which might have attracted inflammatory cells towards the wound (Al-Henhena et al, 2011). Increased cellular proliferation may be due to the mitogenic activity of the plant extract, which might have significantly contributed to healing process. Early dermal and epidermal regeneration in treated rats also confirmed that the extract had a positive effect towards cellular proliferation, granular tissue formation and epithelialization (Karodi Jadhav, Rub, & Bafna, 2009).

In the present study, it was noticed that the neovascularization period for both 75% and 100% doses of Aloe vera (*Aloe vera*) leaf aqueous extract is short compared to the control groups. The findings indicate that Aloe vera had a prominent effect on the rate of wound contraction. The report coincides with the findings of Tsuchiya, et al. (2006) who reported that Aloe vera leaf gel promote the wound healing mechanism The percentage of wound closure was high in both 75% and 100% doses of Aloe vera (*Aloe vera*) leaf aqueous extract. This may be due to

the presence of active compounds present in Aloe vera and also substances like enzymes, glycoproteins, growth factors, vitamins and minerals.

CONCLUSION

It is concluded that 100% aqueous Aloe vera extract is the most effective among the four concentrations. This is supported by the histopathological examination. Furthermore, 100% is comparable with the wound healing activity of Mupirocin 2%

RECOMMENDATIONS

Based on the findings of the researchers on the wound healing activity of the aqueous leaf extract of Aloe vera (*Aloe vera*), here are the researchers' recommendations for the enhancement of this study.

1. Conduct a preclinical study to test the plant's efficacy when formulated into a dosage form.
2. Formulate the extract into a dosage form.

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