Global Journal of Pharmacology 10 (1): 23-30, 2016
ISSN 1992-0075

DOI: 10.5829/idosi.gjp.2016.10.01.102183

# Wound Healing Potentials of Aqueous Pineapple (*Ananas comosus*) Extract - a Preliminary Report

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**Abstract:** The wound healing potentials of aqueous pineapple extract (*Ananas comosus*) were evaluated in eighteen (18) adult male albino rats (152±1.6g) randomized into 3 groups following a 2cm full-thickness skin incision induced on their dorsum. Group A wounds were treated twice daily with aqueous pineapple extract (APE)(n=6), B -silver sulphadiazine (SSD) (n=6) and C- phosphate buffer saline (PBS) (n=6). All wounds were evaluated conventionally with gross and histologic wound healing indices. Wound edge oedema, hyperaemia and exudation were prominent in all the groups between days 0 and 1 of the study. Wound edge oedema was, significantly less ($p<0.05$) on day 2 in APE (30%) compared with SSD(83%) and PBS(100%), (APE< SSD< PBS). Wound hyperaemia was markedly less, on days 2 and 3, though not significant ($p > 0.05$) (APE< SSD< PBS). Wound exudation was significantly less ($p<0.05$) on day 2 in APE compared to SSD and PBS (APE<SSD< PBS). Wound contraction was significantly higher ($p<0.05$) between day 2 and 3 in APE group compared with SSD and PBS groups. Marked contraction was also observed between days 4 and 6 in APE and SSD groups (SSD> APE> PBS). The histologic changes observed on day 7 and 14 showed significant ($P< 0.05$) amount of Type 1 collagen, blood capillary regression and wound epithelialization in the APE and SSD groups compared with PBS group. Results of this study showed that aqueous pineapple extract possesses wound healing potentials compared to silver sulphadiazine and recommended for wound management in poor resource settings of third world countries.

**Key words:** Pineapple Extract · Rats · Silver Sulphadiazine · Wound Healing

## INTRODUCTION

Wound repair is a biologic process influenced by several mediatory factors that often determine its rapidness [1]. Body response to injury (physical, chemical or thermal) involves inflammatory, debridement and repair phases that results in tissue morphologic and functional restoration [2, 3]. The rate at which wound heals is a measurable index of interest in wound research [4]. Wound healing agents and methods such as foam dressings, alginates, hydrocolloids, hydrofibres, hydrogels, transparent films, negative pressure wound therapy, growth factors, skin substitutes, high pressure water irrigation, topical collagen products, topical insulin and topical antioxidants, stem cells and gene therapy have been developed and evaluated clinically with varying results [5-9]. The cost of these materials and methods often limit their availability and access by the poor majority in developing economies of Africa and Asia [10, 11].

In the United State of America, a conservative estimation of cost of wound care in the human population is $50 billion per year [12]. This was estimated as 10 times more than the annual budget of the World Health Organization as at the time of the report [13].

Wound cases constitute a high percentage of clinical caseloads among animals and humans in developing countries of the world [14, 15]. This has necessitated the search for locally available, inexpensive, effective and natural wound healing products. Wine, vinegar, egg yolk and boiling oil are some natural products that have been used in ancient times [16]. In more recent times, the wound healing effect of honey has also been reported [4, 17- 20].

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The Pineapple plant (*ananas comosus*) has been widely used as a therapeutic plant in several cultures [21]. Pineapple belongs to the family of *Bromeliacea*. The plant contains the enzyme complex, bromelain, which reduces pain and swelling [22, 23], facilitate wound drainage [24-26] and stimulate production of collagenase which digests wound necrotic tissue [27, 28] and also increases the production of interferon-alpha and interferon-gamma that aid fibroblast mobilization to wound site [29]. It also contains carotenoids and polyphenols which are powerful antioxidants [30]. Despite these effects of pineapple bromelain, there is dearth of information in literature on the use of aqueous pineapple extract for wound management purposes. Hence this study aimed at investigating the wound healing potentials of aqueous pineapple extract using gross and histologic wound healing parameters and to relate the individual and cumulative results obtained from treatment groups to the benefits of applying aqueous pineapple extract on skin wound.

## MATERIALS AND METHODS

Eighteen (18) adult male albino rats weighing 152±1.6g were used for the study. Approval of the Animal Use and Care Committee (ACURECC) of the University of Ibadan, Nigeria was obtained before commencement of the study. The rats were sourced from a local breeding unit, housed at the Experimental Animal Housing Unit of the Faculty of Veterinary Medicine, University of Ibadan, in well aerated individual cages and exposed to a 12 hour light/dark cycle, 21 ± 0.5°C temperature and 23 - 30% relative humidity, provided with rat diet and clean water *ad libitum.*

**Anaesthesia and Wound Creation:** Each rat was anaesthetized with an intramuscular injection of 5% Ketamine (35.0 mg/kg) and 2% Xylazine (5.0 mg/kg) via the quadriceps group of muscles as earlier described [31]. Following anaesthesia, the dorsum (back) of each rat was prepared for aseptic surgery and a full-thickness midline incision wound was induced on the back (Thoraco-lumber area) of each rat each with size 15 scalpel blade fixed on a sized 3 Bird Parker blade holder.

**Preparation of Aqueous Pineapple Extract (Crude Bromelain):** Fresh pineapple was obtained from the local market and identified to be smooth Cayenne Pineapple at the Department of Botany, University of Ibadan. The fruit was washed with distilled water, dried and peeled off. Seventy (70) grams of the fruit was homogenized using a blender and processed with40ml of phosphate buffer saline as previously described [32].

**Research Design and Treatment Application:** A simple randomized controlled study approach was adopted. Following wound creation, rats were randomized into 3 treatment groups. Group A (n=6): Aqueous pineapple extract (APE), 0.5 ml twice daily; Group B (n=6): Silver sulphadiazine (SSD) (Positive control) applied to wound twice daily as specified and Group C (n=6): Phosphate buffer Saline (PBS) (Negative control) 0.5 ml applied to wound twice daily until wounds healed (between days 0 to 14).

**Evaluation of Gross Wound Healing Indices:** Each animal was evaluated from day 0 to 14. Wound exudation, wound edge oedema and wound surface hyperaemia were observed and scored. Wound contraction was evaluated using Vanier caliper as earlier described [4].

**Evaluation of Histologic Wound Healing Indices:** Tissues obtained (entire wound region excised in-depth with liberal margins of the surrounding skin including underlying connective tissues above the external fascia of the dorsal muscles) from 3 animals per group following euthanasia with Ketamine (70 mg/kg) and xylazine (15 mg/kg) at day 7 and 14, stored in (10%) formalin, cut by microtome into 5 $\mu$m thick sections and stained with hematoxylin-eosin were used for the study. Levels of granulation tissue, vascularization, fibro elastic tissue and epithelialization were evaluated as previously described [33] with semi-quantitative four-point scale scoring system. The scoring was done by two pathologists who worked independently. A third pathologist collated the results from the previous two and re-investigated possible area of contradiction.

**Data Analysis:** Data generated were presented either in percentages or mean ± standard deviation and compared with Analysis of Variance (ANOVA) at 5% confident level.

## RESULTS

**Wound Edge Oedema:** Wound edge oedema was prominent in the three groups from day 0 to 1, but was significantly less ($p<0.05$) in APE (30%) on day 2 than in SSD (83%) and PBS (100%) groups with the trend being APE< SSD< PBS (Table 1).

Table 1: Percentages of animals in the three groups that showed wound edge oedema in the first seven days

| | APE | | SSD | | PBS | |
|---|---|---|---|---|---|---|
| Days | N | % | N | % | N | % |
| 0 | 6 | 100 | 6 | 100 | 6 | 100 |
| 1 | 6 | 100 | 6 | 100 | 6 | 100 |
| 2 | 2 | 33 | 5 | 83 | 6 | 100 |
| 3 | 0 | 0 | 4 | 67 | 3 | 50 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 |

N= number of animals that showed wound edge oedema

%= percentage of animals that showed wound edge oedema

APE= aqueous pineapple extract; SSD= silver sulphadiazine; PBS= phosphate buffer saline

Table 2: Percentages of animals in the three groups that showed wound surface hyperaemia in the first seven days

| | APE | | SSD | | PBS | |
|---|---|---|---|---|---|---|
| Days | N | % | N | % | N | % |
| 0 | 6 | 100 | 6 | 100 | 6 | 100 |
| 1 | 6 | 100 | 6 | 100 | 6 | 100 |
| 2 | 3 | 50 | 4 | 67 | 4 | 67 |
| 3 | 1 | 17 | 1 | 17 | 2 | 33 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 |

N= number of animals that showed wound surface hyperaemia

%= percentage of animals that showed wound surface hyperaemia

APE= aqueous pineapple extract; SSD= silver sulphadiazine; PBS= phosphate buffer saline

Table 3: Percentages of animals in the three groups that showed wound surface exudation in the first seven days

| | APE | | SSD | | PBS | |
|---|---|---|---|---|---|---|
| Days | N | % | N | % | N | % |
| 0 | 6 | 100 | 6 | 100 | 6 | 100 |
| 1 | 6 | 100 | 6 | 100 | 6 | 100 |
| 2 | 6 | 17 | 6 | 33 | 6 | 33 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 |

N= number of animals that showed wound surface exudation

%= percentage of animals that showed wound surface exudation

APE= aqueous pineapple extract; SSD= silver sulphadiazine; PBS= phosphate buffer saline

Table 4: Rate of wound contraction across the three groups

| | Mean incision length [± standard deviation] in centimeters | | |
|---|---|---|---|
| Days | APE | SSD | PBS |
| 0 | 2.00±0.00 | 2.00±0.00 | 2.00±0.00 |
| 1 | $1.90{\pm}0.03^{a}$ | $1.78{\pm}0.12^{b}$ | $1.92{\pm}0.08^{c}$ |
| 2. | $1.50{\pm}0.14^{a}$ | $1.63{\pm}0.08^{b}$ | $1.77{\pm}0.08^{c}$ |
| 3. | 1.27±0.23 | 1.33±0.14 | 1.58±0.16 |
| 4. | 0.85±0.31 | 0.80±0.13 | 1.42±0.23 |
| 5. | 0.65±0.24 | 0.40±0.17 | 1.13±0.31 |
| 6. | 0.38±0.34 | 0.03±0.08 | 0.93±0.26 |
| 7. | 0.15±0.23 | 0.00±0.00 | 0.72±0.22 |
| 8. | 0.00±0.00 | 0.00±0.00 | 0.60±0.26 |
| 9. | 0.00±0.00 | 0.00±0.00 | 0.43±0.12 |
| 10. | 0.00±0.00 | 0.00±0.00 | 0.27±0.06 |
| 11. | 0.00±0.00 | 0.00±0.00 | 0.13±0.12 |
| 12. | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| 13. | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| 14. | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |

Difference of values with superscript a is significantly higher than the differences between values with superscripts b and c

APE= aqueous pineapple extract; SSD= silver sulphadiazine; PBS= phosphate buffer saline

Table 5: Histologic wound healing indices on day 7 and day 14 across the three groups

| Histologic parameters | APE | SSD | PBS |
|---|---|---|---|
| Day 7 | | | |
| Granulation tissue | $1.00{\pm}0.00^{a}$ | $1.00{\pm}0.00^{a}$ | $2.67{\pm}0.58^{b}$ |
| Vascularization | $1.00{\pm}0.00^{a}$ | $0.67{\pm}0.58^{b}$ | $2.33{\pm}0.58^{c}$ |
| Fibro-elastic tissue | $1.00{\pm}0.00^{a}$ | $1.33{\pm}0.58^{a}$ | $0.00{\pm}0.00^{b}$ |
| Epithelialization | $3.00{\pm}0.00^{a}$ | $3.00{\pm}0.00^{a}$ | $1.00{\pm}0.00^{b}$ |
| Day 14 | | | |
| Granulation tissue | 0.00±0.00 | 0.33±0.58 | 0.67±0.58 |
| Vascularization | 0.00±0.00 | 0.00±0.00 | 0.33±0.58 |
| Fibro-elastic tissue | 2.00±0.00 | 2.00±0.00 | 2.00±0.00 |
| Epithelialization | 3.00±0.00 | 3.00±0.00 | 3.00±0.00 |

Superscripts a, b and c indicate value of heterogeneous subsets

APE= aqueous pineapple extract; SSD= silver sulphadiazine; PBS= phosphate buffer saline

**Wound Surface Hyperaemia:** Wound hyperaemia was prominent in all the groups on days 0 and 1 but was markedly less on day 2 (APE= 50%; SSD= 67%; PBS= 67%) and day 3 (APE= 17%; SSD= 17%; PBS= 33%) with the trend being APE< SSD< PBS (Table 2).

**Wound Surface Exudation:** Wound exudation was prominent in all the groups from day 0 to 1 but was significantly less ($p<0.05$) in the APE (17%) at day 2 compared to SSD (33%) and PBS (33%) with the trend being APE< SSD< PBS (Table 3, Revise highlighted figures after correction of Table 3).

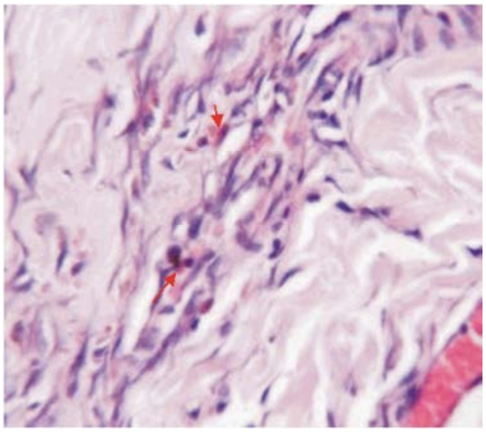

Plate 1: Tissue histologic section of aqueous pineapple extract group at day 7. Take note of the granulation tissue with few blood capillaries [red arrows]. [Haematoxylin-eosin stain, x400]

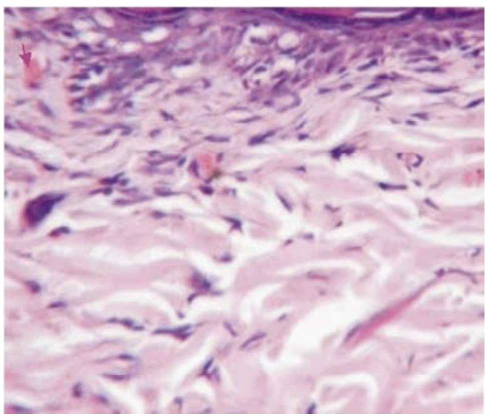

Plate 2: Tissue histologic section of silver sulphadiazine [positive control] at day 7. Take note of the granulation tissue with few blood vessels [red arrows]. [Haematoxylin-eosin stain, x400]

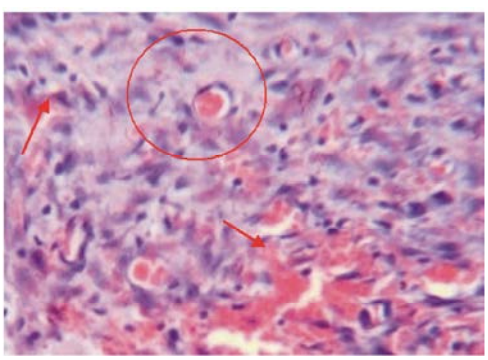

Plate 3: Tissue histologic section of phosphate buffer group at day 7. Take note of the many blood capillaries [red arrows] and capillary loops [red circle] of granulation tissue. [Haematoxylin-eosin stain, x400]

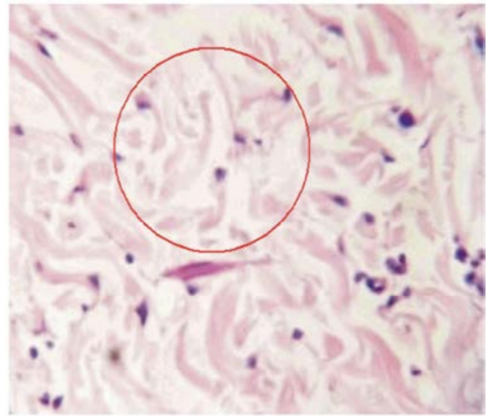

Plate 4: Tissue histologic section of aqueous crude extract group [study group] at day 14. Take note of the fibro elastic tissue [red circle] with no blood capillaries. [Haematoxylin-eosin stain, x400]

**Wound Contraction:** Wound contraction was gradual in all the groups from day 0 to 14. It was however significantly higher ($p<0.05$) between day1 and 2 in APE group compared with SSD and control (PBS). Marked contraction was also observed between day 4 and 6 of APE and SSD groups with the contraction trend being SSD> APE> PBS (Table 4).

**Granulation Tissue:** The amount of granulation tissue (type III collagen) at day 7 was significantly higher ($p<0.05$) in PBS than in APE and SSD. The amount of granulation tissue at day 14 was not significant in all the groups. (Table 5 and Plates 1-6).

**Vascularization [Angiogenesis]:** Blood vessels regressed faster in APE and SSD than in PBS group with the level of vascularization significantly higher ($p<0.05$) in PBS than in APE and SSD at day 7. Level of vascularization at day 14 was not significant ($p<0.05$) in all the groups (Table 5 and Plates 1- 6).

**Fibro Elastic Tissue:** Amount of fibro elastic tissue at day 7 was significantly higher ($p<0.05$) in APE and SSD than in PBS. There was no significant difference ($p<0.05$) in levels of fibro-elastic tissue at day 14 in all the groups (Table 5 and Plates 1- 6).

**Epithelialization:** Level of epithelialization at 7th day was significantly higher ($p<0.05$) in APE and SSD than in PBS. There was no significant difference ($p<0.05$) in levels of epithelialization on day14 across the groups (Table 5, Plates 7-9).

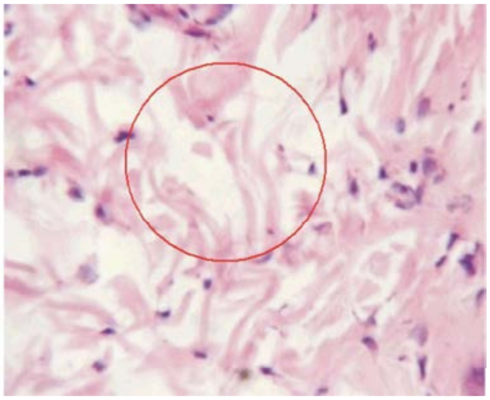

Plate 5: Tissue histologic section of silver sulphadiazine group [positive control] at day 14. Take note of the fibro-elastic tissue [red circle] with no blood capillaries. [Haematoxylin-eosin stain, x400]

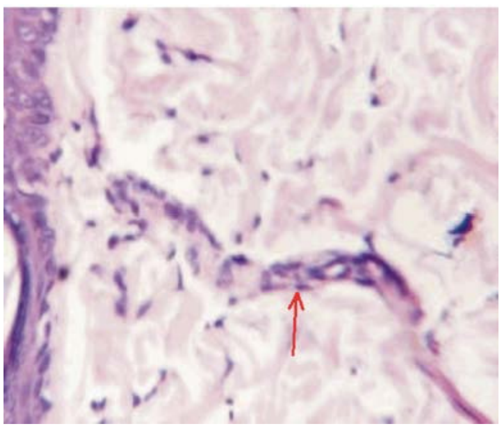

Plate 6: Tissue histologic section of phosphate buffer group [negative control] at day 14. Take note of the scanty granulation tissue [red arrow] with no blood capillaries. [Haematoxylin-eosin stain, x400]

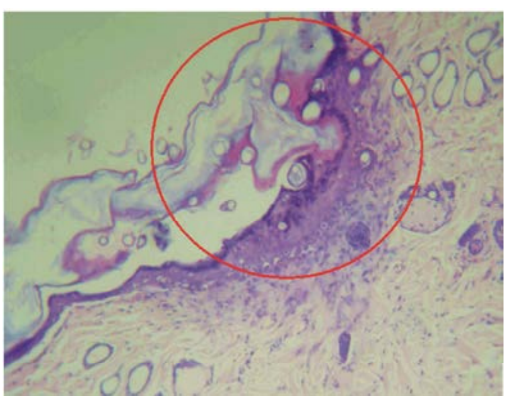

Plate 7: Tissue histologic section of aqueous pineapple extract group [study group] at day 7 showing complete epithelialization [red circle]. [Haematoxylin-eosin stain, x400]

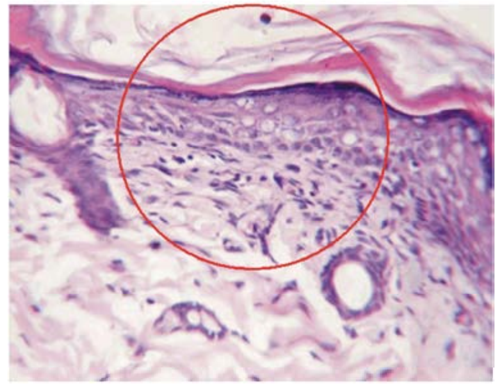

Plate 8: Tissue histologic section of silver sulphadiazine group [positive control] at day 7 showing complete epithelialization[red circle]. [Haematoxylin-eosin stain, x400]

Plate 7: Tissue histologic section of phosphate buffer group [negative control] at day 7 showing partial epithelialization. [Haematoxylin-eosin stain, x400]

## DISCUSSION

Results of this study showed that aqueous pineapple extract has impressive healing potentials on skin wound studied. Silver sulphadiazine was used as a positive control in this study for its previously confirmed wound healing efficacy [34, 35]. Phosphate buffer saline was used to prevent fermentation and stabilisation of aqueous pineapple extract pH as established in previous studies [32, 36].

**The Wound Healing Indices:** Gross (oedema, hyperaemia, exudation, wound contraction) and histologic (granulation tissue, angiogenesis, fibro-elastic tissue, inflammation, epithelialization) evaluated in this study are conventional to wound healing studies [4, 33].

Acute wound oedema, surface hyperaemia and exudation observed in this study were severe on day days 0 and 1 (tables 1, 2 and 3).These indices were indications of tissue response to injury [37]. Wound edge oedema was significantly less ($p<0.05$) on day 2 in the aqueous pineapple extract group (APE)[30%] compared to silver sulphadiazine group (SSD)(83%) and phosphate buffer saline group (PBS)(100%)(APE< SSD< PBS)(Table 1). This anti-oedema effect of crude pineapple extract has been associated with its bromelain content (22, 38&39). Pineapple bromelain reduces oedema by reducing the cleavage of high molecular weight kinin (HMWK) into bradykinin, a potent stimulator of vascular permeability [40].

Wound hyperaemia was markedly less on days 2 and 3 (APE< SSD< PBS) (Table 2). Bromelain has been postulated to markedly reduce hyperaemia in a previous study in some dogs (Boxer breeds) with face, chest and arm bruises [41].

The significantly less wound exudation ($p<0.05$) observed in this study (Table 3) was previously observed with bromelain on wounds [42]. Aqueous pineapples extract bromelain has been hypothesized to influence the production of bradykinin, a substance that increases vascular leakage and engorgement during inflammation [43].

Wound contraction was gradual in all the groups from day 0 to day 14. It was however significantly higher ($p<0.05$) between day 2 and 3 in APE group compared with SSD and control (PBS) (Table 4). This could be traced to the influence of bromelain on the production of plasmin which breaks down the tiny clots blocking small blood capillaries which facilitates microcirculation of the wound area and hence mobilization of fibroblasts [37]. Some of the fibroblast population transform to myofibroblasts, which is mainly responsible for wound contraction [44].

As wound matures, granulation tissue (consisting mainly of collagen type III) undergoes transformation into extracellular matrix (consisting mainly of collagen type I), the number of blood vessels is reduced and the amount of fibroelastic tissue increases [45]. At day 7 of this study, extent of granulation tissue transformation was significantly higher ($p<0.05$) in APE and SSD than in PBS. This was traceable to the bromelain content of aqueous pineapple extract which has been shown to facilitate wound permeability [46]: a factor essential for granulation tissue formation [26].

On day 7, regression of blood capillaries was significantly higher ($p<0.05$) in APE and SSD than in PBS meaning that wound maturity was greater in the APE and SSD (Table 5; Plates 1-6). This corroborated the observation in a study on the debriding effect of bromelain [47].

The amount of fibro- elastic tissue on day 7 was significantly higher ($p<0.05$) in APE and SSD than in PBS which indicates that type I collagen was more abundant in APE and SSD than in PBS treated wounds (Table 5; Plates 1-6). This result is in agreement with that obtained by Aiyegbusi *et al.* [30] who observed that aqueous pineapple extract stimulate proliferation of tenoblasts (a form of fibroblast in tendons).

Level of epithelialization in samples collected on day 7 was significantly higher ($p<0.05$) in APE and SSD than in PBS (Table 5, Plates 7-9). Epithelialization, one of the proliferative processes of wound repair, starts with mobilization and migration of epithelial cells at the margin of the wound [48]. In full-thickness wound, the epidermis only resurfaces from the margins after adequate granulation tissue has formed [49, 50]. This suggested that granulation tissue filled up the wound defect of APE and SSD treated wounds faster than those PBS treated wounds.

## CONCLUSIONS

From the result of this study we concluded that aqueous pineapple (*Ananas comosus*) extract has remarkable wound healing effects on skin wounds of wistar rats and therefore recommended for skin wound management especially in poor resource settings of Africa and Asia.

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