

Unripe rind and pulp of *Ananas comosus* accelerate wound healing in diabetic wistar rat

Running title: *Ananas comosus* and wound healing

ABSTRACT

Objective: One of the sequelae of diabetes mellitus is chronic foot ulcer. This study investigated wound healing propensity of the pulp and rind of *Ananas comosus* (a widely cultivated fruit with many health benefits) in diabetic wistar rats.

Methods: The excisional wounds of twenty four adult wistar rats with induced diabetes in four groups of six each were dressed with the pulp powder (PPD), pulp extract (PED), rind powder (RPD) and rind extract (RED) of *A. comosus* daily till healed. Also the mean wound contraction rates were calculated from the wound areas every three days. Granulation tissue was biopsied from an animal per group on day 3,6 and 9 for histopathological evaluation. Each of these groups had a corresponding equal number control group with similar procedures performed.

Results: The mean wound contraction rates of the rind pulp diabetic (RPD) and rind extract diabetic (RED) groups paralleled those of their respective control group (RPC, REC) as they were not significantly different. Also amongst the diabetic groups, the rind of *A. comosus* demonstrated greater wound healing capability over the respective pulp counterpart (PPD, PED) as evidenced by significantly higher mean wound contraction rates. Histopathologic features of the granulation tissues and scars of the diabetic groups were similar to those of the control groups.

Conclusion: Although, both the rind and pulp of unripe *A. comosus* either in extract or powder formulation were shown in this study to possess excellent healing potentials in diabetic wounds, the rind appeared to be better off and may be a viable alternative to the wound dressing materials currently in use in the management of diabetic wounds / ulcers.

Key words: *Ananas comosus* pulp and rind, diabetes, wound healing.

1.Introduction

From time immemorial, medicinal plants have been the sources of a large array of chemical compounds of diverse biological functions [1]. These chemical compounds of plant extraction mediate their effects on the human body through mechanisms that are similar to those in conventional drugs hence the similarity between the synthetic drugs and herbal medicines [2]. *Ananas comosus* (pineapple) of the Bromeliaceae family is a herbaceous perennial plant that is widely cultivated with many health benefits being attributed to it [3]. Diabetes is one of the largest global non-communicable diseases with life-changing and life-threatening complications. According to the International Diabetes Federation 2015 report; about 415 million (8.8 % prevalence rate) people aged between 20-79 years are diabetic globally with over 14 million from the African Region and the estimate for Nigeria said to be more than 1.56 million [4]. Global mortality due to diabetes was 5 million this accounted for 14.5 % of global-all cause mortality among people in this age group. One of the complications of diabetes with significant contribution to diabetic death and non-trauma related lower limb amputation is diabetic foot ulcer (DFU). It is a chronic non healing wound due to hyperglycaemic induced peripheral angiopathy and neuropathy [5]. Many materials such as honey [6], biopads [7], sofratulle [8] and hydrocolloids [9] are being used as dressing materials in the management of DFU with varied outcome. Diabetes mellitus is a chronic non-communicable disease that requires life-long management that could be a financial burden. Management of DFU in an African setting is big financial challenge to the patients thus for most patients the use of the synthetic materials earlier enumerated is very limited for reasons of availability, affordability and accessibility. Consequently, the wound healing capabilities of many plants in animals with induced diabetes mellitus have been studied. Such plants include *Lycopersicon esculentum* (tomato) [10], *Carica papaya* (pawpaw) [11], *Musa paradisiaca* (plantain) [12] and *Mangifera indica* (mango) [13]. All these have given promising but yet to be translated results to humans. The availability, affordability and accessibility of the herbal extracts obtained from these naturally occurring and widely cultivated plants to the patients will be of immense benefit and offer a great relief in the management if successfully translated. Many health benefits of *Ananas comosus* (pineapple) have been documented in the literature, there is however, a dearth of information on its benefit or otherwise as a dressing material in diabetic wounds. Thus there is a need to elucidate such benefits if they exist hence the justification for this study.

2. Materials and Method

2.1 Plant Materials

2.1.1 Collection and identification of plant materials

Unripe *Ananas comosus* (pineapple) fruits were procured from a cash crop market situated in Oje Ibadan, South West Nigeria. Identification and authentication was done at the Botany Department, University of Ibadan. Specimens were deposited at the herbarium of the Department.

2.1.2 Preparation of powders and extracts

After washing the fruits, they were subsequently peeled to obtain both the rind (outer cover) and the pulp (the juicy component). These were separately dried at room temperature with filtered air. Thereafter, each was milled by means of an electric blender to obtain fine powdery substances. Three hundred and fifty grammes of each was used for subsequent analyses.

Phytochemical analyses

Using the analytical techniques previously described by Harborne [14], Boham and Kocipai [15], Obadoni and Ochuko [16], Ejikeme et al [17]; the phytochemical analyses of *A.comosus* were done with 8g each of the rind and pulp.

Ethanollic extractions

Two hundred and fifty grammes (250 g) each of the powdered pulp and rind were used for the ethanollic extractions with 100 % ethanol. The resultant extracts were in gel form and the pulp yield was 11.70 % and that of the rind being 14.98 %. They were stored under optimal conditions till use.

2.2 Animals

Forty eight healthy inbred adult female wistar rats with a weight range of 150-210 g sourced from the central animal house of the College of Medicine, University of Ibadan were used for the study. They had a two week acclimatization in a well ventilated and illuminated environment with conducive ambient temperature. For the duration of the study, they had liberal standard rat diet and water. The animals were closely monitored and those that developed features of sepsis were withdrawn from the study. The animals were handled in

accordance to the guidelines as prescribed by the ethical conduct of animal research of the University of Ibadan. Also, the principles of laboratory animal care as contained in the 8th edition (2011) of the Guide for the Care and Use of Laboratory Animals by the National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals were observed. (National Academies Press (US); 2011. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK54050/> doi: 10.17226/12910).

2.3 Design of the Experiment

Using diabetes and dressing material as criteria for group allotment, the animals were randomly allotted into eight groups of six each with four of the groups being control and four were diabetic. These groups were as stated below:

- 1.Pulp powder control (PPC)
- 2.Pulp extract control (PEC)
- 3.Rind powder control (RPC)
- 4.Rind extract control (REC)
5. Pulp powder diabetic (PPD)
6. Pulp extract diabetic (PED)
7. Rind powder diabetic (RPD)
- 8.Rind extract diabetic (REC)

2.4 Induction of Diabetes Mellitus

The pre induction fasting blood sugar levels were estimated with single touch glucometer (ACCUCHECK®, Roche Diagnostics, Germany) using the blood obtained from the tails of the rats. A value range of 48.5-75 mg/dl was obtained and served as baseline. Based on the outcome of previous studies by us, a single dose of 100 mg/kg body weight of alloxan monohydrate dissolved in normal saline and administered intraperitoneally was used to induce diabetes mellitus. A fasting blood sugar level above 200 mg/dl obtained 72 hours post induction was considered diabetic.

2.5 Wound Creation and Management

For the purpose of wound creation, each rat was sedated with intramuscular ketamine hydrochloride (120 mg/kg). Thereafter, the dorsolateral skin was cleansed with savlon antiseptic liquid and a 2 cm by 2 cm full thickness skin about 1.5 cm from the vertebral column was excised.

2.6 Wound Management and Data Collection

The pulp powder, pulp extract, rind powder and rind extract was used as wound dressing material for the respective paired group- PPC, PPD; PEC, PED; RPC, RPD; and REC, RED. Wound dressing was done daily. However, before change of dressing, wound size estimation was done by taking dimensions along two perpendicular plane. The values obtained were used to derive the contraction rates in percentages and this was repeated every 3 days.

Granulation tissue biopsy was taken from a member of each group on day 3,6 and 9. These samples were processed for histological evaluation using Haematoxylin and Eosin stain. These slides were used for evaluation of wound healing in terms of cellularity, angiogenesis, fibroplasia and collagen synthesis. The excision of granulation tissue served as the exit point for such animals and the wounds of remaining animals in all the groups were allowed to heal. The resultant scars were similarly processed for light microscopy. Falling off of the eschar

without any residual wound indicated the endpoint of complete epithelization and the days required for this connoted the duration of healing.

2.7 Data Analysis and Processing

The numerical aspects of the results were analyzed with Statistical Package for the Social Sciences (SPSS) version 23 and expressed as percentages, means plus standard deviation of means (SD). The student t-test was used for inter group comparison and level of significance was set at $p < 0.05$

3 RESULTS

Both the pulp and rind of *Ananas comosus* tested positive for anthraquinones, terpenoids, alkaloids, flavonoids, tannins and saponins however, the pulp had greater content of flavonoids than the rind. Cardiac glycosides were not detected in either of them (Table 1). All the diabetic groups recorded weight loss ranging from 13- 21 % seven days following induction of diabetes, the largest being the PPD group. A repeat measurement of the body weight on day 14 revealed weight gain that cut across all these groups. However, none of these group attained the pre-induction mean weight (Table 2). On day 3 of study, three of the diabetic groups namely PPD, PED and RED had negative wound contraction rates in essence, their wound sizes increased rather than reduced. By day 6, only groups PPD and RED still had negative mean wound contraction rates though reduced. Three days later i.e day 9, all the diabetic groups had positive contraction rates with that of the RED being over 50 %. By day 12, the two rind diabetic groups i.e. RED and RPD had respective mean contraction rate of 82.94 ± 0.26 and 71.1 ± 7.4 while the pulp diabetic groups were below 50%. Thus the rind diabetic groups achieved wound healing earlier than PED and PPD with the latter being the last to achieve healing (Table 3).

Mean wound contraction rates comparisons

Comparisons of the mean wound contraction rates between each control group with its diabetic counterpart revealed that the control was significantly higher for PPC vs PPD on day 3, 6 and 12; PEC vs PED on day 3 and REC vs RED on days 3 and 6. Comparisons amongst the diabetic groups revealed that the mean contraction rates for RPD were significantly higher than those of the PPD on day 3, 9 and 12. While those of the RED higher than values for the PPD group on days 9 and 12. The mean wound contraction rates of the RPD were significantly higher than those of the PED group on day 3 and 12 and the RED group had significantly higher value than RPD only on day 3 (Table 4).

Granulation tissues

On day 3, the histology of the granulation tissue biopsied from a representative of each group revealed; (i) predominance of inflammatory cells in both PPC and PPD but more intense in

the latter group. (ii) new blood vessels and very scanty cells in group PEC while PED showed much cellular infiltration and macrophages. (iii) multilayered squamous cells suggestive of epithelial migration were observed in both PEC and RPD. (iv) RPC showed considerable cellular infiltration with neovascularization. However, spindle shaped cells suggestive of fibroblasts were observed in RPD and it was less cellular in comparison to RPC. (v) Considerable macrophages were observed in REC while paucity of cells but very abundant fibrillary structures were observed in RED (Plate1). On day 6, the light microscopy slides prepared from representative granulation tissues showed predominance of collagen fibrils and paucity of cells in groups PPC, PPD, PEC and PED. Also stratified squamous epithelium was observed in group PPD. Numerous cells and new blood vessels were observed in groups RPD and REC. While group RED was characterized by new epithelium, fibroblasts with collagen fibrils (Plate 2). The main features of day 9 granulation tissues were fibroblast and collagen fibrils (Plate 3). Sections of the wound scars showed well defined epidermal and dermal layers in all the groups with abundance of collagen fibrils but very sparse cells (Plate 4)

Table 1. Phytochemical analyses of unripe pulp and rind of *Ananas comosus*

Anthraquinones		Terpenoids		Alkaloids		Flavonoids		Tannins		Saponins		Cardiac Glycosides	
Pulp	Rind	Pulp	Rind	Pulp	Rind	Pulp	Rind	Pulp	Rind	Pulp	Rind	Pulp	Rind
+	+	+	+	+	-	++	+	+	+	+	+	-	-

+ (scanty), ++ (moderate), and - (absent)

Table 2 Mean fasting blood sugar levels and mean body weight

Parameter	PPC	PPD	PEC	PED	RPC	RPD	REC	RED
Pre induction sugar level (mg/dl)	NA	60.5	NA	51	NA	48.5	NA	52.25
Post induction sugar level (mg/dl)	NA	265	NA	270.5	NA	260.5	NA	258.25
Pre induction weight (g)	153.6±3.13	159.6±7.50	157.25±4.19	205.4±50.24	156± 3.31	168.4±10.26	154.25±2.5	162.8±7.69
Post induction-7 days (g)	138.5±12.02	125.5±31.20	149.5±7.79	177±43.86	150± 2.83	146±10.44	149.67±2.31	141.25±10.10
Post induction-14 days (g)	158± 4.24	153.5±12.02	167.5±7.79	204.5±16.26	161.67±7.5	157.5±23.34	169± 2.83	149.5±0.71

NA= Not applicable. The control groups were not induced; the weights were itemized just to show the trend.

Following the induction of diabetes, the blood sugar levels were markedly elevated in all the four diabetic groups. The post induction blood sugar level was significantly higher than the respective pre induction value ($P < 0.05$).

Table 3. Interval mean values of wound contraction rates in percentages (%)

Group	Day 3	Day 6	Day9	Day 12	Day 15	Day18	Day21	Day24
PPC	14.22±7.92	38.7±23.8	50±2 3.6	81.2±11. 5	87.6±9.4 1	95.8±7.37	***	
PPD	-38.32±15.25	- 11.08±25.2	20.46 ±3.71	25.79±5. 24	36.91±9. 1	55.8±7.37	67.54±3.54	78.32±17.8
PEC	27±16.31	19.3±11.1	52.9± 20.2	71.61±16 .3	84.5±12. 7	93.9±10.09	97.8± 8.33	***
PED	-28.55±13.87	1.57±18.67	27.19 ±13.7	41.5±9.3 4	64.5±11. 4	78.9±12.09	83.8±7.37	95.12±6.87
RPC	20.12±12.75	20.73±17.2 8	45.34 ±9.39	81.58±10 .47	86.45±6. 45	93.5±3.7	98.64±5.4	***
RPD	18.32±11.10	13.6±10.2	33.96 ±4.75	71.1±7.4	86.45±6. 45	93.5±3.7	96.64±5.2	***
REC	20.76±12.77	25.8±8.9	83.6± 4.0	89.8±1.8	93.5±0.8	98.4±0.5	***	
RED	-29.97±44.45	- 3.75±20.46	50.95 ±21.0	82.94±0. 26	84.5±12. 7	86.45±6.45	93.9±9.09	***
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The asterisks connote that the wounds had healed. As expected the wounds of the control groups healed earlier than those of the diabetic groups. On day 3 and 6, some of the diabetic groups had negative values as wound contraction rates this implied that the wounds expanded rather than contracted.

Table 4: Intra and Inter group comparisons of wound mean contraction rates

Compared groups	Day 3 mean rates	Day 6 mean rates	Day 9 mean rates	Day12mean rates
PPC vs PPD	14.22±7.92*	38.7±23.8*	50±23.6	81.2±11.5*
PEC vs PED	-38.32±15.25	-11.08±25.2	20.46±3.71	25.79±5.24
RPC vs RPD	27±16.31*	19.3±11.1	52.9±20.2	71.61±16.3
REC vs RED	-28.55±13.87	1.57±18.67	27.19±13.78	41.5±9.34
PPD vs PED	20.12±12.75	20.73±17.28	45.34±9.39	81.58±10.47
PPD vs RPD	18.32±11.10	13.6±10.2	33.96±4.75	71.1±7.4
PPD vs RED	20.76±12.77*	25.8±8.9*	83.6±4.0	89.8±1.8
PED vs RPD	-29.97±44.45	-3.75±20.46	50.95±21.04	82.94±0.26
PED vs RED	-38.32±15.25	-11.08±25.2	20.46±3.71	25.79±5.24
RPD vs RED	-28.55±13.87	1.57±18.67	27.19±13.78	41.5±9.34
REC vs RED	-38.32±15.25*	-11.08±25.2	20.46±3.71*	25.79±5.24*
PPD vs PED	18.32±11.10	13.6±10.2	33.96±4.75	71.1±7.4
PED vs RPD	-38.32±15.25	-11.08±25.2	20.46±3.71*	25.79±5.24*
PED vs RED	-29.97±44.45	-3.75±20.46	50.95±21.04	82.94±0.26
RPD vs RED	-28.55±13.87*	1.57±18.67	27.19±13.78	41.5±9.34
REC vs RED	18.32±11.10	13.6±10.2	33.96±4.75	71.1±7.4
PPD vs PED	-28.55±13.87*	1.57±18.67	27.19±13.78	41.5±9.34*
PED vs RPD	-29.97±44.45	-3.75±20.46	50.95±21.04	82.94±0.26
RPD vs RED	18.32±11.10*	13.6±10.2	33.96±4.75	71.1±7.4
REC vs RED	-29.97±44.45	-3.75±20.46	50.95±21.04	82.94±0.26

In comparing mean wound contraction rates, some of them were of statistical significance ($P < 0.05$); such are indicated with asterisk ().*

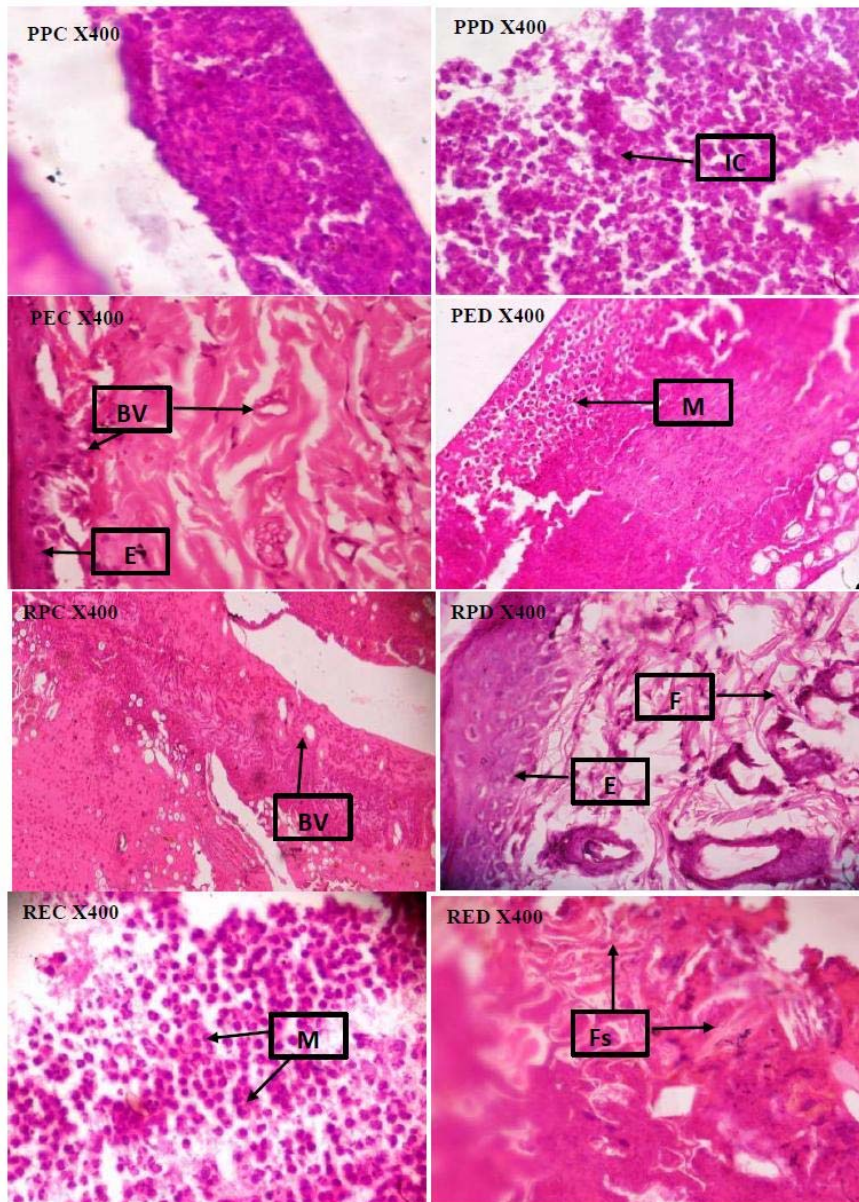


Plate 1. Granulation tissue at Day 3 (H & E).

PPC (Pulp powder control), PPD (Pulp powder diabetic), PEC (Pulp extract control), PED (Pulp extract diabetic), RPC (Rind powder control), RPD (Rind powder diabetic), REC (Rind extract control), RED (Rind extract diabetic), BV (Blood vessel), IC (Inflammatory cells), E (stratified squamous epithelium), F (Fibroblast), Fs (Fibrillary structures) and M (macrophages).

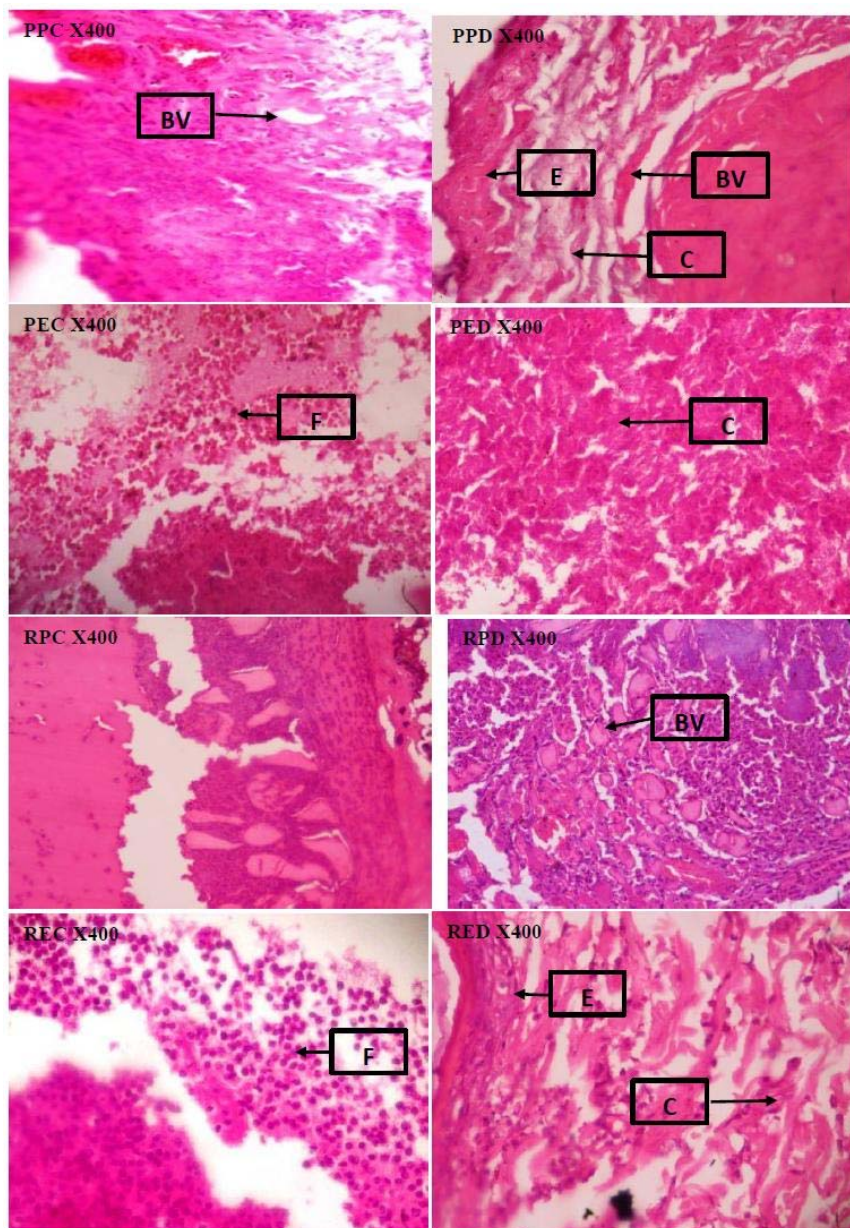


Plate 2. Granulation tissue at Day 6 (H & E).

PPC (Pulp powder control), PPD (Pulp powder diabetic), PEC (Pulp extract control), PED (Pulp extract diabetic), RPC (Rind powder control), RPD (Rind powder diabetic), REC (Rind extract control), RED (Rind extract diabetic), BV (Blood vessel), C (Collagen), E (stratified squamous epithelium) and F (Fibroblast).

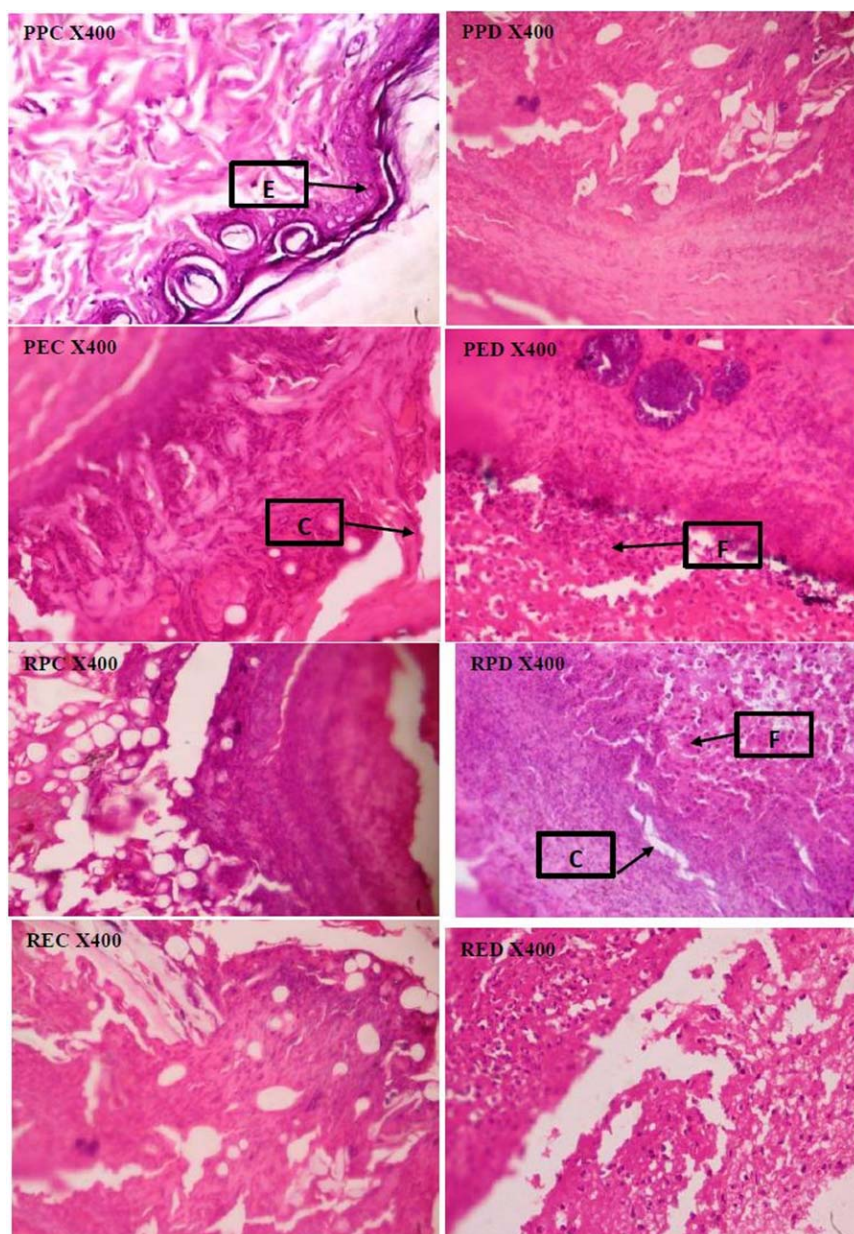


Plate 3. Granulation tissue at Day 9 (H & E).

PPC (Pulp powder control), PPD (Pulp powder diabetic), PEC (Pulp extract control), PED (Pulp extract diabetic), RPC (Rind powder control), RPD (Rind powder diabetic), REC (Rind extract control), RED (Rind extract diabetic), C (Collagen), E (stratified squamous epithelium) and F (Fibroblast).

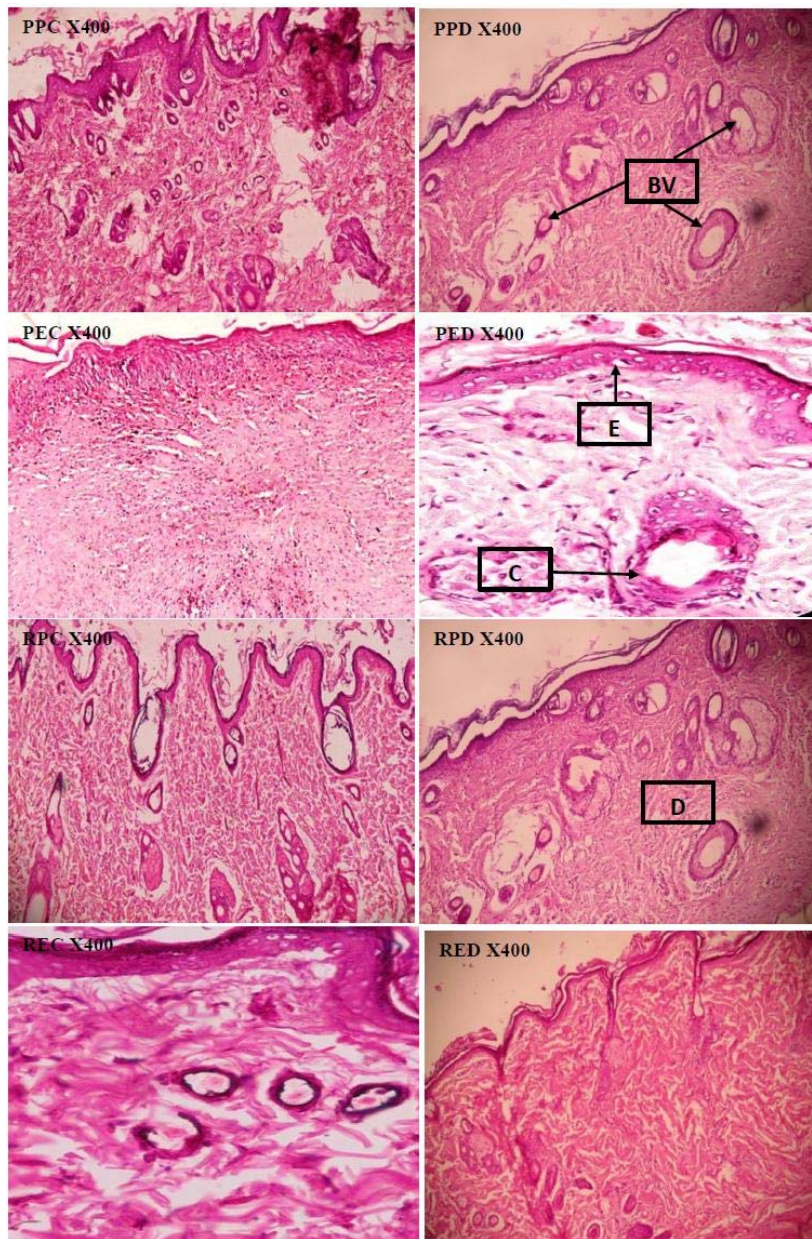


Plate 4. Sections from the wound scars (H & E).

PPC (Pulp powder control), PPD (Pulp powder diabetic), PEC (Pulp extract control), PED (Pulp extract diabetic), RPC (Rind powder control), RPD (Rind powder diabetic), REC (Rind extract control), RED (Rind extract diabetic), BV (Blood vessels), C (Collagen), D (Dermis), E (stratified squamous epithelium) and F (Fibroblast).

DISCUSSION

Weight loss was recorded in both control and diabetic groups on day 7 of the study, this was due to the metabolic response that usually follows surgical trauma. Following trauma, there is increased synthesis of catabolic hormones such as insulin, growth hormone and thyroid hormones. These hormones increase substrate (carbohydrate, protein and fat) catabolism with attendant weight loss. The chemical inflammatory response caused by the instillation of alloxan monohydrate might explain why the recorded weight loss was more pronounced in the diabetic groups. The catabolism triggered by these hormones usually wanes in the absence of the initiating factor(s) provided there is no co-morbidity; this could explain the subsequent weight gain observed on day 14. In this study, wound contraction rate was one of the parameters used to evaluate healing. It was observed that three of the diabetic groups (PPD, PED and RED) had negative mean wound contraction rates on day 3, this trend persisted till day 9 after which they became positive. The values subsequently becoming positive could be suggestive of the reversal of the inhibitory effect of diabetes on wound healing. It might thus be inferred that the pulp and rind of *Ananas comosus* are capable of accelerating wound healing in diabetic rats. The pattern of the contraction rate on day 12; wherein the mean rates for the two diabetic rind groups (RPD and RED) were remarkably higher than those of the pulp groups i.e. PPD and PED was suggestive of the rind being a better accelerator of the wound healing process than the pulp. Diabetes mellitus is known to retard wound healing; the non-significant differences between the mean wound contraction rates of the rind powder control (RPC) and its diabetic counterpart (RPD) recorded throughout the entire duration of the study could be highly suggestive of the rind powder of *A. comosus* being anti diabetic. For the rind extract groups (REC and RED), the differences in the contraction rates became insignificant on days 9 and 12, this might suggest that the extraction altered the anti-diabetic property of the rind of *A. comosus*. It might be concluded that using the rind of *A. comosus* in the powdery formulation as a wound dressing material yielded better result than its extract. For the pulp powder groups (PPC and PPD), the differences in the mean contraction rates were largely significant thus the antidiabetic potential of the pulp powder of *A. comosus* is likely to be very low. When the rates for the pulp extract control (PEC) were compared with those of the diabetic group (PED); the observed differences were statistically insignificant on day 6, 9 and 12. Thus it could be inferred that the extract of the pulp of *A. comosus* possess better anti-diabetic property than the powder hence the result of the wound healing evaluation. Thus if the pulp of *A. comosus* is to serve as a wound dressing material, using it in as extract formulation will produce a better wound healing outcome. This assertion appeared to be contradicted by the outcome of the comparison between groups PPD and PED which did not confer any advantage of the extract over the powder of the pulp since the comparisons of the two groups (PPD vs PED) were of no significance. However, the rind powder appeared to accelerate the healing process when compared with the pulp powder (RPD vs PPD) thus the powder of the rind of *A. comosus* might be a better option over the pulp powder in the management of diabetic wounds. The results of this study also demonstrated that the extract and powder of the rind (RED and RPD) accelerated wound healing faster than the respective pulp group (PED and PPD) in diabetic rats. Amongst the diabetic groups, the histology of the granulation tissue of the RPD group showed the earliest feature of collagen synthesis as evidenced by the presence of fibroblasts on day 3 this could be suggestive of the rind being able to accelerate wound healing. Healing appeared to be delayed in the pulp diabetic groups as evidenced by the more intense inflammatory cell infiltration when compared with the respective control. This observation was likely due to the induced diabetes mellitus. The predominance of collagen fibrils and paucity of cells observed in the light microscopy of the granulation tissues from the pulp groups on day 6 was due to the initial delay of the healing

process occasioned by diabetes, this observation was in concordance with the documented mean wound contraction rates for the same period i.e. day 6. Thus the pulp of *A. comosus* was able to promote healing in diabetic rats though with an initial delay. The neovascularization (angiogenesis) seen in the RPD as well as epithelialization and collagen synthesis seen in the sections from the RED further buttressed the assertion made earlier that pineapple rind was superior to the pulp in terms of promoting wound healing in a diabetic setting. The histologic features of the scars from both the control and the diabetic groups being similar clearly demonstrated the efficacy of the pineapple pulp and rind as dressing materials in the management of diabetic wounds.

Bromelain an isolate of *A. comosus* is a cysteine protease which has been demonstrated to be a potent enzymatic debridement of necrotic tissue from wounds and ulcers [18]. In a review of the potential benefits of bromelain, Rathnavelu et.al.[19] noted that it was being widely administered as a wound healing agent amongst its numerous benefits. Also bromelain has been documented to enhance healing of episiotomy wound in women following vaginal delivery [20]. A patented commercially available extract of *A. comosus* known as Tarcorin[®] is said to contain proteolytic enzyme, glycine, proline, glutamine and arginine. These amino acids are essential for collagen synthesis. Tarcorin has been shown to promote wound healing by modulating the expression of cytokines and growth factors such as tumour necrosis factor, transforming growth factor β and metalloproteinase 2 [18]. This may thus be the mechanism through which *A. comosus* promotes wound healing. Quite a number of studies have postulated that the phytochemicals present in medicinal plants promote wound healing [21-23]. Our study revealed the presence of phytochemicals such as flavonoids, anthraquinones, alkaloids and tannins in both the pulp and rind of *A.comosus*; thus these phytochemicals may be responsible for the ability of *A. comosus* to enhance wound healing in diabetic rats. The results of the phytochemical screening were similar to those of Prakoso et.al [24] who demonstrated that *A.comosus* cream promoted healing in wounds infected with methicillin resistant staphylococcus aureus. The global annual production of pineapple is estimated to be over 14 million tons [25] and the rind constitutes 10 % of the weight of the fruit [26]. This implies that pineapple contributes significantly to agricultural waste worldwide and may thus have a negative impact on the environment. Thus finding a potentially beneficial use for the rind as elucidated by the findings of this study will reduce the environmental burden that the rind constitutes.

Conclusion The results of this study have demonstrated that the rind of *A.comosus* promoted wound healing and did it better than the pulp. This finding is of great economic significance when the environmental and human health hazards that the rind constitutes. Thus finding economic and health friendly use for the rind of *A.comosus* will be of tremendous benefit.

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