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EVALUATION OF WOUND HEALING, ANTI-BACTERIAL AND ANTI-OXIDANT PROPERTIES OF *ANNONA SENEGALENSIS* HYDRO-ETHANOL ROOT BARK EXTRACTS

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ABSTRACT

Background: Traditional healers claims that *Annona senegalensis* possess wound healing ability. This study was designed to assess the wound healing, anti-oxidant and antibacterial potential of *Annona senegalensis* ethanol root bark extract using rats. **Materials and Methods:** Extraction was by cold maceration. In excision wound study, the healing effect of 20% and 10% *Annona senegalensis* ointment were compared with 5% povidone-iodine and paraffin wax. Post wounding, rate of wound contraction, epithelialization, antimicrobial activity and wound histology were studied. In the incision wound model, 20% and 10% of plant extract at 400mg/kg body weight were administered to rats post wounding. The hydroxyproline, hyaluronic acid, total protein, superoxide dismutase, catalase, malondialdehyde assays and wound tensile strength were assessed. **Results:** *Annona senegalensis* extract treated groups showed faster wound healing, with good antioxidant and antibacterial properties. **Conclusions:** ASEE showed good wound healing effect and antibacterial activities, which were comparable to those of povidone-iodine.

KEYWORDS

Annona senegalensis, Antibacterial, Antioxidant and Wound healing.

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INTRODUCTON

A wound is the interference of cell arrangement, anatomical formation, and functional continuity of living tissue[1]. Activities such as physical damage, chemical irritations, thermal burns, microbial infection, or immunological insult to the tissues can lead to wound formation[2]. Wounds are classified as closed or open wound (Based on the underlying cause of wound / nature of wound creation) and acute or chronic wound (Based on the physiology of wound healing)[3].

Wound healing is essentially a survival mechanism which aims to maintain a normal anatomical structure and function of the body[4]. Presence of wound stimulates the immune system to initiate and execute wound healing naturally. However, a wound can be invaded by microorganisms which suppress the immune system leading to delayed, unhealed or chronic wound[5]. Therefore, wound treatment is very necessary in order to hasten wound healing[6]. Some measures taken in wound repair includes the use of topical and systemic antimicrobial, antiseptics, anti-inflammatory agents, etc[2]. However, the limitations in the use of these orthodox medicinal approach are their cost-intensive implications and some elicit side effect[6]. In view of the above shortcomings, there is need for cheaper and safe complementary substance (efficiently found in medicinal plants) that could promote wound healing[7,8].

Annona senegalensis popularly identified as wild custard apple or wild sour-sop is one of the plants extensively used for wound management in south-eastern Nigeria[9]. It posses anti-venomous[10], anti-trypanosome[11], anti-inflammatory[12], analgesic[13], antimalarial[14] and antimicrobial[15]. Despite the folkloric claims that *A. Senegalensis* possesses a wound healing ability, there are no scientific reports authenticating its ethnomedicinal use in the management of wounds based on the available literature. Research shows that, plants extracts which contain some phytochemical agents like; flavonoids, alkaloids and tannins stimulate wound healing through their antibacterial and antioxidant activities[16], since the phytochemical screening of *A. Senegalensis* shows the presence of these metabolites, it may possess wound healing and antibacterial properties. The objective of this study, therefore, was to evaluate the antibacterial and wound healing properties of ethanolic root extract of *A. Senegalensis.*

MATERIAL AND METHODS

The experimental procedures employed in this study were approved by the Ethics Committee of the University of Nigeria, Nsukka and is in line with the guide to the care and use of animals in research and training of the University.

Plant Collection and Identification

Fresh leaves and roots of *A. Senegalensis* were collected in May 2018, from Udenu LGA of Enugu State, Nigeria. The leaves were identified by a taxonomist, at the International Centre for Ethnomedicine and Drug Development (Inter CEDD), Aku Rd, Nsukka, Enugu State, Nigeria. A voucher specimen was deposited at the Inter CEDD herbarium with a voucher number Inter CEED/064.

Extraction

Two kilograms (kg) of fresh root bark of *A. Senegalensis* were cut into smaller sections, air-dried at room temperature for two weeks and then pulverized into a coarse powder. Extraction was by cold maceration using 500g of the pulverised root bark in 80% ethanol for 48h period. The filtrate was obtained using a Whatman's filter paper, then concentrated using hot air oven set at 40ºC. The extract was measured and the percentage yield calculated.

Preparation of Ointment

10% and 20% w/w Herbal ointment were prepared separately employing the method of Okore *et al*[17]. They were aseptically transferred into sterile cream tubes with seal, and preserved in the refrigerator until use.

Animals

A total seventy (70) females albino rats weighing between 200g and 250g were obtained in June 2018, from the animal unit, in Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were housed for 2 weeks at the Department of Veterinary Surgery, University of Nigeria, Nsukka. To acclimatize before commencing the experiment. Food and water were provided *ad libitum* throughout the experimental period.

Determination of Minimum Inhibitory Concentration (MIC)

Seven concentrations of ASEE namely 500mcg/ml, 250mcg/ml, 125mcg/ml, 62.5mcg/ml, 31.25mcg/ml, 15.625mcg/ml, and 7.8mcg/ml were prepared. The MIC of the extract was determined by pipetting 50mcg each of the seven concentrations mentioned above into five wells each and mixed with equal volume nutrient broth in a microtitre plate. Specifically, 0.1 ml of standardized inoculum (1-2 × 107 CFU/ml) was added to each tube. The tubes were incubated aerobically at 37°C for 18-24 hr.

Two control wells were maintained for each test batch. These included antibiotic control (well-containing extract and the growth medium without inoculum) and organism control (the well containing the growth medium, physiological saline and the inoculum). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tubes were regarded as MIC[18].

Assessment of wound healing effect of *A. Senegalensis* ointment on excision wound

A total of forty (40) female albino rats were randomly assigned to 4 groups of 10 rats per group were anaesthetized by intramuscular injection of 10 and 50mg/kg BW of xylazine and ketamine hydrochloride respectively. The hairs on their back were shaved off and the area sterilised with 70% alcohol then traced out with a circular rubber seal and a marker, and excised using surgical scissors[19]. After the wound creation, they were assigned into four treatment groups (n=10) and treated from A-D with 20%, 10% ASEEO, povidone-iodine, and paraffin wax respectively.

Percentage excision wound contraction

Their wound diameters were measured at 3, 6, 9, 12, 15, 18 and 21 days post wounding (DPW) using a transparent meter rule, and the percentage wound contraction calculated using the method of Ezike *et al*[20].

Excision wound epithelialization

Period of wound epithelialization was calculated using the method of Nayak *et al*[21]. Their mean day for complete healing with epithelium covering the entire wound and hairs appear in the wound site was calculated using the procedure described by Okoli *et al*[22].

Excision wound microbial assay

Post wounding wound sites were left untreated for 24 hours to establish infection. Wound swabs were taken from all the rats at days 1, 3, 7 and 14 (prior to topical treatment). Swabs were inoculated in brain heart infusion (BHI) broth (Oxide) and incubated at 37°C for 24 hours aerobically. The broth cultures were observed for microbial growth (cloudiness/turbidity), on observation of turbidity, a loop full of the broth culture was sub-cultured on blood agar and incubated at 37°C for 24 hours. Isolates of different colonial types present were purified on fresh media, incubated and then used for identification following standard biochemical methods[23].

Histologic Examination of excision wound tissue

Three rats in each group were sacrificed by an overdose of chloroform anaesthesia on days 7 and 14. The wound sites were excised, leaving a 5mm margin of normal skin around the edges of the wound. Histology was carried out following the method of Bancroft and Gamble[24]. Semi-quantitative evaluation of the wound slides for re-epithelialization, polymorph nuclear leukocytes, tissue macrophages (TM), fibroblasts, neovascularization, collagen formation and Type 1 collagen was done using a subjective scoring method on a 5point scale from 0 to 4 by 2 independent observers blinded to the treatment protocol[25].

Healing effect of *A. Senegalensis* on laparotomy incision wounds of rats

Thirty female albino rats were randomly assigned into 3 groups A to C (n=10), and anaesthetized with 10 and 50mg/kg BW of xylazine hydrochloride and ketamine hydrochloride. The ventral abdomen were shaved and disinfected with methylated spirit. A 4cm length laparotomy incision was made into the abdominal cavity using sterile scalpel blade size 24. The incision site was subsequently sutured with chromic catgut 2/0 using simple continuous suture pattern for the muscle while the skin was sutured with silk size 2/0 using simple interrupted suture pattern. They were dosed orally with 20% and 10% of 400 mg/kg ASEE and 1ml/kg BW of sterile distilled water once daily for 14 days, from A-C respectively. The flow technique described by Morton and Malone[19], was used to determine wound tissue breaking strength at day 10 PW, after suture removal at day 8PW.

Assay of biochemical parameters in incision wound tissue

Wound biopsy specimens were gotten from three rats in each group at days 7 and 14 pw. The specimens were macerated immediately using mortar and pestle and used for assaying the following biochemical parameters using these following methods namely; superoxide dismutase (SOD) by Magwere *et al*[26], Malondialdehyde assay

(MDA) by Ohkawa *et al*[27], catalase (CAT) by Beers and Sizer[28], hydroxyproline (Hyp) by Sharma *et al*[29], Hyaluronic acid level by Lowry *et al*[30] and total protein (TP) as described byTietz[31].

STATISTICAL ANALYSIS

Data obtained were summarized as mean ± standard error of the mean (SEM). Mean values of wound breaking strength, percentage wound contraction, wound epithelialisation, time for complete healing and biochemical parameters assayed in the different groups were compared using one-way Analysis of Variance. Duncan multiple range tests were used to separate variant means. $P < 0.05$ was considered significant.

RESULTS

EXTRACTION

ASEE was a brown oily and sticky substance, with aromatic smell. The percentage yield was 11.2% w/w material.

Minimum Inhibitory Concentration (MIC)

The MIC for *Staphylococcus spp, Pseudomonas aeruginosa, Bacillus spp, Escherichia coli* and *Klebsiellaspp* were 500mcg, 500mcg, 500mcg, 500mcg and 7.8mcg respectively (Table No.1).

Percentage wound contraction of excision wounds of rats

The percentage rate of wound contraction (12.3%) of Group C (povidone-iodine treated group) on day 3 pw was significantly (P<0.05) higher than those of groups A, B and D (10%, 20% ASEEO and soft paraffin wax treated group respectively), from days 6 to day 21 of the study. However no significant difference (p>0.05) was observed between percentage wound contraction of groups A and B but both groups showed significantly (P<0.05) higher rate of wound contraction when compared with groups A and D. However on days 9, 12 and 21 the percentage rate of wound contraction of rats in group A was significantly (p<0.05) higher than those of group D. Also group D, however, showed the least rate of wound contraction throughout the study period (Table No.2).

Epithelialization time of excision wounds

Epithelialization occurred significantly (P<0.05) faster in animals in group B (18.00 ± 0.41) and C (16.00 ± 0.41) than those in group A (21.50 ± 0.65) and D (26.75 ± 0.85). While group D had a significantly (P<0.05) longer epithelisation time (Table No.3).

Qualitative estimation of bacterial isolate in excision wound

On day 1 post wound treatment, groups A and D had few Staphylococcal growths and moderate Bacillus growth while group B had both few Bacilli and Staphylococcus growth, whereas group C had few Bacilli and moderate Staphylococcus growth. On day 3 Bacillus and Staphylococcal growth became few in all the groups except for group D which maintained moderate bacillus and staphylococcal growths. On days 7 and 14 bacterial growth were absent in groups A, B and C except for a few bacillus growths observed in group A. However group D maintained few growths of Bacillus and Staphylococcal infections on day 7 with only few Bacillus observed in the same group on day 14.

Histologic analysis of excision wound tissue

On day 7, both fibroblast and epithelial cells were found to be absent in all the groups however there were moderate levels of inflammatory cells in the treated groups while the control had the highest level of inflammatory cells as shown in (Table No.5) and (Figure No.1). On day 14, groups B and C showed moderate and high levels of collagen and epithelial cells respectively, while groups A and D had moderate levels of both fibroblast and epithelial cells. However, few inflammatory cells were observed in all the groups on day 14 (Figure No.2).

Effects of ASEE on biochemical parameters in incised wounds of rats

The hydroxyproline (HyP) level throughout the study period showed a dose-dependent increase from A to C progressively and there were significant (P<0.05) difference between the groups, with group A significantly (P<0.05) having the highest level of HyP.

On days 7 and 14, the hyaluronic acid levels of ASEE treated groups (A and B) were significantly (P<0.05) higher than that of the control. Hyaluronic acid also showed a concentration-dependent increase in the treated groups.

At day 7 pw, the total protein (TP) in wound biopsy of animals in the 20% ASEE treated group (A) was significantly (P<0.05) higher compared to the

control but the TP level of the control did not differ from group B while on day 14, TP of group A and B which were dose-dependent increased significantly (P<0.05) compared to group C which decreased significantly on day 14 (Table No.6).

Antioxidant effects of ASEE on incision wounds of rats

On days 7 and 14 there are significant different between the MDA level of groups A, B and C however no significant difference was observed when group A was compared with C. group A showed the least level of MDA compared to MDA levels of group B and C and distilled water (group B). However when compared statistically no significant (P>0.05) difference existed between A and B while there was significant (P<0.05) difference when group A was compared with group C.

On day 7 pw, there were no significant (P>0.05) differences in the levels of SOD of the treated and control groups. However on day 14, group A significantly (P<0.05) had the highest SOD level while that of group C was significantly (p<0.05) the least. The SOD levels of ASEE treated groups in this study were also dose-dependent. At days 7 and 14 the CAT level throughout the study period showed a dose-dependent increase from A to C with group A significantly (P<0.05) having the highest level of CAT while group C had the least (Table No.7).

Wound tissue breaking strength in rats post infliction of the incision wound

The result showed that group A (20% ASEE treated groups) significantly (*P*< 0.05) had higher wound breaking strength when compared with group B (10% ASEE) and control (Table No.8).

DISCUSSION

According to Al-Henhena *et al*[32], excision and incision wound are the standard model for evaluation of wound healing potential of plants, and as such were used in this study. In excision wound model, healing was allowed to take place through second intension healing, which is characterized by wound contraction and re-epithelialization[33]. A significant increase in percentage wound contraction, shorter re-epithelialization and shorter wound healing time observed, implies a faster rate of wound healing in group B (20% ASEEO) and C (5% Povidone-iodine). This is due to the ability of groups B and C to enhance collagen deposition in healing tissue. This was further established by the histologic examination of wound tissues. Enhancement of collagen deposition by the extract was observed to occur better at a higher concentration, leading to a difference in epithelisation time between the two extract treated groups (A and B). Wound contraction is a result of the action of myofibroblast and occurs throughout the healing period while epithelialisation involves the proliferation and migration of epithelial cells towards the centre of the wound, leading to the formation of new epithelial covering over the wound[34]. Moderate level of Bacillus and Staphylococcus spp were the only bacterial isolated from the wound site throughout the study period and were completely wiped out at the end of the study. Previous study of the phytochemical analysis of *A. senegalensis* shows the presence of tannins, terpenoids, essential oils, alkaloids, and flavonoids. This gives it the antimicrobial potentials and hence inhibition of other bacterial growth[15]. Collagen is composed of amino acid and hydroxyproline, and it supports and strengthens extracellular tissue.

In incision wound, a dose-dependent increase in total protein and hydroxyproline level were obtained in wound tissue harvested from rats. This is a reflection of their higher collagen content, which possibly enhanced epithelisation and contraction. This was further confirmed by the dose-dependent increase in tensile strength. Tensile strength has commonly been associated with the organization, content, and physical properties of the collagen fibril network[35]. To achieve effective wound healing, low level of reactive oxygen species (ROS) and oxidative stress is required, and there should be a balance between oxidative stress and antioxidant[36]. SOD and catalase functions as an antioxidant enzyme that destroys the peroxides (NO^-, NO_2, $ONOO^-$, H_2O_2, OH^- and O_2^-) and play a significant role in providing antioxidant protection to an organism[37]. In this study lesser MDA concentrations and associated higher SOD and CAT concentration in the same ASEE treated groups (A and B) were observed. Increase in SOD and CAT concentration leads to a decrease in the

concentration of (MDA) lipid peroxidative enzyme and vice versa[38]. The above observation, therefore, suggests that the extract has a high antioxidant property which invariably assisted in accelerating rate of wound contraction, epithelialization and decreased wound microbial contamination observed.

Table No.1: Minimum inhibitory concentration (MIC) of ASEE on 5 micro- organisms

S.No	Micro-organism spp	MIC
1	*Staphylococcus spp*	500mcg
2	*Pseudomonas aeruginosa*	500mcg
3	*Bacillus spp*	500mcg
4	*Escherichia coli*	500mcg
5	*Klebsiellaspp*	7.8 mcg

Keys: spp: species; mcg: Microgram

Table No.2: Result of percentage wound contraction of excision wounds of rats (Mean ± SEM, n=10)

Groups	Day 3 (Mean ± SEM)	Day 6 (Mean ± SEM)	Day 9 (Mean ± SEM)	Day12 (Mean ± SEM)	Day 15 (Mean ± SEM)	Day 18 (Mean ± SEM)	Day 21 (Mean ± SEM)
A(10% ASEEO)	-12.25±1.84^{a}	2.00±2.00^{a}	18.00±0.91^{a}	36.25±3.01^{a}	45.00±2.88^{a}	62.25±3.57^{a}	76.00±2.04^{a}
B(20% ASEEO)	1.00±1.91^{b}	18.50±2.25^{b}	35.75±1.49^{b}	58.75±0.48^{b}	81.00±2.79^{b}	94.00±6.00^{b}	100.00±0.00^{b}
C (povidone iodine)	12.25±1.65^{c}	23.75±1.75^{b}	36.75±1.79^{b}	61.25±1.75^{b}	84.50±1.65^{b}	100.0±0.00^{b}	100.00±0.00^{b}
D (paraffin wax)	-8.25±2.95^{a}	-1.00±1.91^{a}	8.00±1.63^{c}	17.00±2.16^{c}	27.00±2.44^{a}	35.50±2.84^{a}	46.00±2.82^{c}

Keys: ASEEO: *A. Senegalensis* ethanolic extract ointment; SEM: standard error of mean
^{abc} indicate a significant difference in means at $P<0.05$, across a column

Table No.3: Wound epithelialization time of rats treated with ASEEO, povidone-iodine and soft paraffin wax

S.No	Groups	Epithelialization time in days (Mean ± SEM)
1	A (10% ASEEO)	21.50 ± 0.65^{a}
2	B (20% ASEEO)	18.00 ± 0.41^{b}
3	C (povidone iodine)	16.00 ± 0.41^{b}
4	D (paraffin wax)	26.75 ± 0.85^{c}

Keys: ASEEO: *A. Senegalensis* ethanolic extract ointment; SEM: standard error of mean.
^{abc} indicate a significant difference in means at $P<0.05$, across a column

Table No.4: Qualitative estimation of bacterial isolate in excision wound

Days	Groups	Bacterial Isolated	
		Bacillus spp	Staphylococcus spp
1	A (10% ASEEO)	++	+
	B (20% ASEE0)	+	+
	C (Povidone iodine)	+	++
	D (Soft paraffin)	++	+
3	A (10% ASEEO)	+	+
	B (20% ASEE0)	+	+
	C (Povidone iodine)	+	+
	D (Soft paraffin)	++	++
7	A (10% ASEEO)	+	-
	B (20% ASEE0)	-	-
	C (Povidone-iodine)	-	-
	D (Soft paraffin)	+	+
14	A (10% ASEEO)	-	-
	B (20% ASEE0)	-	-
	C (Povidone-iodine)	-	-
	D (Soft paraffin)	+	-

Keys: +++ =High, ++ = moderate, + = few, - =absent

Table No.5: Qualitative Cellular Estimation in excision Wounds of rats

S.No	Days/Groups	Neutrophils	Macrophages	Lymphocytes	Fibroblasts/ Collagen	Epithelial cells
1	DAY 7 A	++	++	+	-	-
2	B	++	+	+	-	-
3	C	++	++	+	-	-
4	D	+++	+	+	-	-
5	DAY 14 A	-	+	++	++	++
6	B	-	+	+	++	+++
7	C	-	+	+	++	+++
8	D	-	+	++	++	++

Keys: +++: High, ++: moderate, +: few, - : Absent

Table No.6: Estimation of biochemical parameters in incised wound sections of rats

S.No	Biochemical parameters	Groups	Day 7 (Mean ± SEM)	Day 14 (Mean ± SEM)
1	Hydroxyproline	A (20% of 400mg ASEE/kg bw)	35.80 ± 0.58^{a}	44.10 ± 0.81^{a}
		B (10% of 400mg ASEE/kg bw)	24.35 ± 0.32^{b}	28.20 ± 1.38^{b}
		C(0.1ml Distilled water /kg bw)	23.15 ± 0.32^{b}	18.50 ± 0.29^{c}
2	Hyaluronic acid	A (20% of 400mg ASEE/kg bw)	35.60 ± 0.126^{a}	29.00 ± 0.69^{a}
		B (10% of 400mg ASEE/kg bw)	28.35 ± 2.17^{ab}	26.30 ± 0.29^{a}
		C(0.1ml Distilled water /kg bw)	21.85 ± 0.08^{b}	21.20 ± 0.29^{b}
3	Total Protein	A (20% of 400mg ASEE/kg bw)	47.70 ± 1.20^{a}	64.00 ± 2.31^{a}
		B (10% of 400mg ASEE/kg bw)	38.35 ± 4.05^{ab}	48.00 ± 1.15^{b}
		C(0.1ml Distilled water /kg bw)	31.25 ± 0.55^{b}	11.20 ± 0.17^{c}

Keys: bw: body weight, ASEE: *A. Senegalensis* ethanolic extract; SEM: standard error of mean; mg: milligram, ml: millilitre, kg: kilogram.

^{abc} indicate a significant difference in means at $P<0.05$, across a column

Table No.7: Wound MDA, SOD and CAT concentrations in rats post incision Wound creation

S.No	Antioxidants	Groups	Day 7 (Mean ± SEM)	Day 14 (Mean ± SEM)
1	MDA	A (20% of 400mg ASEE/kg bw)	17.20 ± 1.10[a]	11.65 ± 0.61[a]
		B (10% of 400mg ASEE/kg bw)	20.10 ± 0.80[a]	14.65 ± 0.66[ab]
		C (0.1ml Distilled water /kg bw)	28.35 ± 1.55[b]	22.40 ± 1.44[b]
2	SOD	A (20% of 400mg ASEE/kg bw)	247.00 ± 12.00[a]	236.50 ± 3.75[a]
		B (10% of 400mg ASEE/kg bw)	224.50 ± 14.50[a]	178.00 ± 1.15[b]
		C (0.1ml Distilled water /kg bw)	205.50 ± 15.50[a]	139.50 ± 3.17[c]
3	CAT	A (20% of 400mg ASEE/kg bw)	66.50 ± 1.50[a]	67.50 ± 1.44[a]
		B (10% of 400mg ASEE/kg bw)	60.00 ± 5.00[ab]	46.00 ± 3.46[a]
		C (0.1ml Distilled water /kg bw)	49.00 ± 1.00[b]	39.00 ± 0.57[b]

Keys: bw: body weight, ASEE: *A. Senegalensis* ethanolic extract; SEM: standard error of mean; mg: Milligram, ml: Millilitre, kg: kilogram.

[abc] indicate a significant difference in means at $P<0.05$, across a column

Table No.8: Wound tissue breaking strength in rats post infliction of the incision wound

S.No	Groups	Tensile Strength (g) day 10
1	A (20% of 400mg ASEE/kg bw)	1210.43 ± 164.43[a]
2	B (10% of 400mg ASEE/kg bw)	738.44 ± 19.74[b]
3	C (0.1ml Distilled water /kg bw)	444.58 ± 24.14[b]

Keys: bw: body weight, ASEE: *A. Senegalensis* ethanolic extract; SEM: standard error of mean; mg: milligram, ml: mill, kg: kilogram.

[abc] indicate a significant difference in means at $P<0.05$, across a column

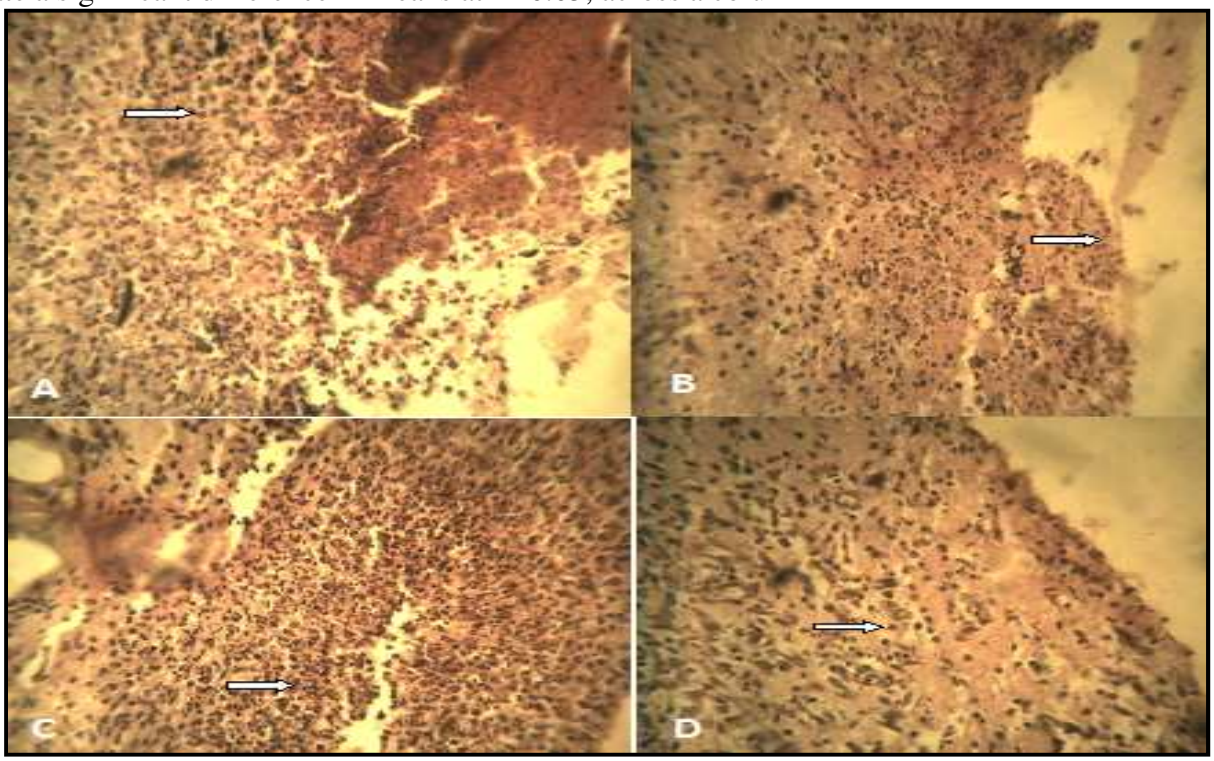

Figure No.1: Photomicrograph of H and E stain of excision wounds of the various experimental groups at day 7 showing areas of eosinophilic exudates (fibrin) and abundant inflammatory cells (few neutrophils, lymphocytes and macrophages) - arrows, H and E x 400

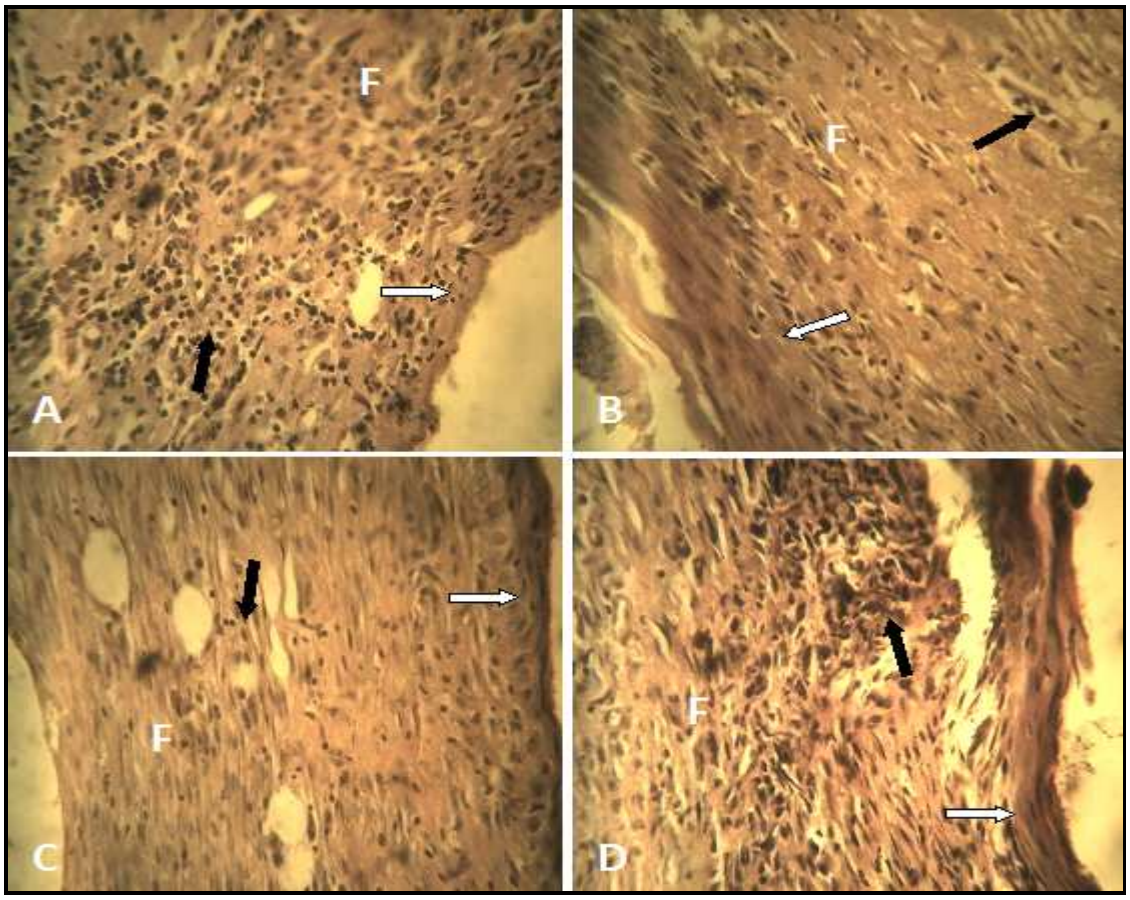

Figure No.2: Photomicrograph of H and E stain of excision wounds of the various experimental groups at day 14 showing areas of fibroplasias (F), inflammatory (mononuclear) cells infiltration (black arrows) and epithelial cells (white arrows). H and E x 400

CONCLUSION

The results of this study have established that the ASEE potentiates wound healing partly by increasing collagen deposition and epithelialization. A dose-dependent healing potential was observed in excision and incision wound studies. Thus, this study validated the folkloric use of this plant (*Annona senegalensis*) in the management of open wounds. However, further studies which would involve cell biology, immunology, and biochemistry to elucidate fully the process of wound healing by *A. senegalensis* root extract is recommended.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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