

Original Research Article

In vivo anti-inflammatory and wound healing properties of *Annona muricata* leaf methanol extract-loaded nanogel

Christian A Alalor^{1*}, Sinodukoo E Okafo¹, Kenneth O Akproko¹, Goodnews I Agare², Benedict B Iwetan²

¹Department of Pharmaceutics and Industrial Pharmacy, ²Department of Pharmacology and Toxicology, Faculty of Pharmacy, Delta State University, Abraka, Delta State, Nigeria

*For correspondence: **Email:** calalor@delsu.edu.ng; **Tel:** +234-8033239656

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Abstract

Purpose: To assess the wound recovery and anti-inflammatory activities of herbal nanogels formulated using *Annona muricata* extract (AME) in Wistar rats.

Methods: The crude methanol extract from *Annona muricata* leaves was processed into a total of seven batches of topical gels (F1 - F7), three batches of nanogels (F1- 1 % AME nanogel, F2 - 2 % AME nanogel, F3 - 3 % AME nanogel), three batches of crude gels (F4 - 1 % AME crude, F5- 2 % AME crude, F6 - 3 % AME crude) and one batch of blank gel (F7). The anti-inflammatory effect was tested using paw edema generated by carrageenan, while the wound restoration effect was assessed on excision-induced rat wounds. Physicochemical evaluation of the gels included pH, viscosity, and spreadability.

Results: The herbal nanogels at 3 %w/w demonstrated significant anti-inflammatory and wound healing effects ($p < 0.05$), surpassing corresponding concentrations of crude *Annona muricata* extract and comparing favorably with the standards. The nanogel formulations exhibited favorable physicochemical characteristics. The optimized formulation, F3 (3 % AME-Nanogel), displayed remarkable anti-inflammatory activity ($p < 0.05$), achieving 100 % edema inhibition after 5 hours and 94.87 % wound closure and epithelialization within 15 days, surpassing the corresponding concentration of crude AME at 92.59 and 90 %, respectively. Enhanced activity of the nanogel could be due to increased solubility and bioavailability of the extract by reducing particle size with a polydispersity index (PDI) of 0.404 and mean size of 73.35 nm.

Conclusion: Extract of *Annona muricata* leaves possesses notable wound recovery and anti-inflammatory activities; the nanogel of *Annona muricata* extract enhanced the wound recovery and anti-inflammatory activities significantly, indicating its potential topical therapy for inflammation and wounds.

Keywords: Anti-inflammatory, Wound healing, Nanogel, Methanol extract, *Annona muricata*

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INTRODUCTION

Phytochemical components exhibit limited absorption due to their high molecular weight and inability to cross lipid membranes [1]. Additionally, over 40% of new bioactive principles

are reported to be poorly water-soluble, leading to slow drug absorption [2]. The majority of plant phytoconstituents face challenges in crossing cell lipid membranes, either due to their significant molecular size or poor water solubility, resulting in restricted absorption and limited bioavailability

[3]. Despite impressive *in vitro* potentials, many phytomedicines exhibit poor *in vivo* activity, rendering them of minimal clinical value [4]. Nanoparticles are materials with at least one dimension measuring between 1 and 100 nanometers (nm). They play a critical function in drug delivery by virtue of their favorable size and large surface area relative to volume ratio. Nanoformulations of phytomedicines have emerged as essential tools to enhance bioavailability, solubility, drug retention time, and delivery, while minimizing toxic effects. This approach has the potential to strengthen the action of phytoextracts, reduce dose and side effects, and improve bioactivity [5].

Annona muricata L., (Family: Annonaceae) known by various names such as soursop, graviola, guanabana, and corossol, is a broad-leaved, evergreen tree distributed widely in tropical areas of Central and South America, Western, Central, and Eastern Africa, and Southeast Asia [6]. Numerous studies highlight the diverse pharmacological activities of *Annona muricata* (*A. muricata*), including analgesic, antioxidant, anti-inflammatory, antibacterial, antihypertensive, antimalarial, antidiabetic, and anticancer properties [7,8].

Inflammation is a complex process involving pain, membrane changes, protein denaturation, and increased vascular permeability. Wound healing is essential for restoring disrupted anatomical continuity and damaged functional status of the skin [9]. The wound restoration process includes coagulation, swelling, granulation tissue formation, matrix formation, connective tissue modification, collagen deposition, and wound strength development [10].

Topical dosage forms such as gels, lotions, ointments, or wound dressings can protect wounds from contamination and speed up the recovery process. Gels, especially for topical therapies, offer an effective and easily manageable option [11]. This study aims to leverage nanotechnology to enhance the delivery of *Annona muricata* extract, maximizing its anti-inflammatory and wound healing potential.

EXPERIMENTAL

Materials

Carbopol 940 (Alpha Lab, Germany), Propylene glycol (Kermel Chemicals, India), Triethanolamine (Dongxiang Zhejiang, China), Carrageenan (Sigma Aldrich, USA), Methanol (Merck, USA), Ethanol (JDH, China), and

Polyvinyl alcohol (Guandong Guanghua Sci-Tech Co. Ltd, China).

Collection of plant material

The fresh leaves of *Annona muricata* were harvested from Abraka, in Ethiopia-East Local Government Area of Delta State on the 4th of July, 2022. The plant sample was identified and validated at the Botany department of the Delta State University, Abraka and assigned DELSUH109 as the voucher number. A sample of the plant was lodged at the herbarium of the Botany department for future reference.

Extraction

The collected leaves were dried in shade for a period of two weeks and comminuted using a mechanical grinder. Two kilograms (2 kg) of the coarse leaves were extracted with 1 L of 80% methanol at a temperature of 65 °C using Soxhlet equipment. The resulting extract was cooled and concentrated using the rotary evaporation method at 40 °C.

Determination of extraction yield

Extraction yield (Y) was calculated using Eq 1.

$$Y (\%) = (W_e/W_d)100 \dots\dots\dots (1)$$

Where W_e is the weight of the concentrated extract while W_d is dry weight of sample.

Phytochemical screening

The preliminary phytochemical screening of the extract of *Annona muricata* was done using standard protocols [12].

Formulation of nanosuspension

The nano-precipitation method was adopted for the preparation of the nanosuspension with slight modification [1]. A 2 g sample of *Annona muricata* extract (AME) was dissolved in 15 mL of hydroalcoholic mixture of 99 % ethanol and distilled water (3:1), then 0.2 mL of Tween 80. The resultant solution was then gradually injected (1mL/min) with a syringe into 40 mL distilled water containing 3.2 % w/v polyvinyl alcohol (PVA) with stirring using a magnetic stirrer at 1000 rpm. A 20 mL quantity of distilled water was used to dilute the solution further and mixed using a magnetic stirrer for 6 h at 500 rpm for evaporation of solvent and nanoparticle formation.

Table 1: Composition of nanosuspension

Ingredient	Quantity
<i>Annona muricata</i> extract (AME)	2 % w/v
Tween 80	0.2 % v/v
99 % Ethanol + distilled water (3:1)	15 % v/v
Polyvinyl alcohol (PVA)	3.2 % w/v

Table 2: Composition of *Annona muricata* nanogels (AME-nanogels)

Ingredient	F1	F2	F3	F4	F5	F6	F7
Nanosuspension of AME (%v/v)	1.0	2.0	3.0	-	-	-	-
Crude AME (%w/w)	-	-	-	1.0	2.0	3.0	-
Carbopol 940 (g)	1	1	1	1	1	1	1
Polyethylene glycol (mL)	2	2	2	2	2	2	2
Ethanol 99 % (mL)	2	2	2	2	2	2	2
Triethanolamine (mL)	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Distilled water to (mL)	100	100	100	100	100	100	100

Particle size analysis of AME-nanosuspension

The polydispersity index and mean particle size of the *Annona muricata* nanosuspension under different homogenization conditions were determined by dynamic light scattering [13], with a Zetasizer ZEN1600 (Malvern Instrument, Worcestershire, UK). Three readings were taken and diameter reported was computed using volume distribution from Gaussian distribution, indicating the width of the particle distribution.

Formulation of gel

The gels were prepared according to the quantities specified in Table 2 below. Carbopol 940 was swelled in distilled water, and then left overnight until it was perfectly hydrated. Propylene glycol and 99 % ethanol were added to the dispersion and stirred.

Triethanolamine was added to neutralize the gel base and stirred until it formed a homogeneous base. The required quantity of nanosuspension of the extract and water (quantity sufficient) was added to the gel base and stirred with a magnetic stirrer for 2 minutes [14].

Evaluation of gel

Determination of pH

The pH of 1 % dispersion of the AME-crude and nanogels was measured using a digital pH meter. The measurement was done in triplicate and the mean measurement was recorded [15].

Viscosity test

The viscosity of the AME-crude and nanogels at 25 °C was obtained using a Brookfield

viscometer at shear rates of 6, 12, 30 and 60 rpm using spindle 4 [16].

Spreadability test

The spreadability measurement was done 48 h after preparation. A 1 g quantity of the AME-nanogel was put in the middle of a glass plate. On top of the gel, a glass plate of the same dimensions (10 x 20 cm) and a 100 g weight were placed. The condition was retained for 1 min, and then the extent of spread was recorded [17].

Preparation of animals

Albino Wistar rats, weighing 150 - 200 g of either sex, sourced from the animal house of the Basic Medical Sciences Faculty at Delta State University, Abraka, were used.

They were acclimatized at 27 °C and relative humidity of 60 % with a 12 h diurnal cycle in the animal house. They were kept in plastic cages and fed consistently with standard rodent diet with water *ad libitum* [18].

Skin irritation test

The furs on the dorsal area of the rats were cleanly shaved, exposing about 6 cm² of skin 24 h before the test. The animals were randomized into seven groups of four rats each and 0.5 g quantities of the formulated gels (F1 to F7) were applied on the shaved area, respectively.

Skin reaction at the site of application was subjectively assessed after 24, 48 and 72 h for any evidence of irritation such as erythema, rashes or oedema [19].

Carrageenan-induced rat paw edema

Animals were fasted for 24 h with unrestricted access to water and randomized into 8 groups (A-H) of four animals in each group. A 0.5 g quantity of the herbal gel formulations (1 % AME-nanogel (F1), 2 % AME-nanogel (F2), 3 % AME-nanogel (F3), 1 % AME-crude (F4), 2 % AME-crude (F5) and 3 % AME-crude (F6)) were respectively applied to the plantar surface of the right hind paw of group A, B, C, D, E and F by gently massaging 50 times with the index finger [20]. A 0.5g quantity of the gel base (control) and methyl salicylate gel (standard) was applied to groups G and H. One hour later, 100 μ L (0.1 mL) of a 1 % suspension of carrageenan in saline was injected into the plantar side of the same right hind paw of rat previously rubbed as explained above. The paw volume measurement was taken immediately after carrageenan injection and at intervals of 1, 2, 3, 4 and 5 hours using a plethysmometer [21]. The relative potency of the herbal gel formulations and drug was computed based on the inhibition (I) of the inflammation using Eq 2.

$$I (\%) = (V_o - V_t / V_o) 100 \dots\dots\dots (2)$$

Where V_o represents the paw volume immediately after the administration of test drugs to the rats) and V_t represents the paw volume at the specific time of measurement.

Wound healing activity of gels

The rats were sedated by inhalational chloroform prior to wound creation. The dorsal fur was shaved, and the wound area was marked on the back of the animals. A linear incision wound having a length of 2.0 cm was created with a surgical blade to the depth of the subcutaneous tissues (0.5 cm deep) under sterile conditions [22]. The rats were randomized into 8 groups (A, B, C, D, E, F, G and H) of 4 rats each. A 0.5 g quantity of the herbal gel formulations (1 % AME-nanogel (F1), 2 % AME-nanogel (F2), 3 % AME-nanogel (F3), 1 % AME-crude (F4), 2 % AME-crude (F5) and 3 % AME-crude (F6)) was respectively applied on the induced wound of all groups. A 0.5 g quantity of the gel base (control) and gentamycin cream (standard) was respectively applied to groups G and H. The gels for all groups were applied once daily until complete closure of wound.

Percent wound contraction and epithelialization time

Wound healing, depicted as percentage wound contraction (C, %) and time of epithelialization,

was noted after total wound healing. Time of epithelialization was monitored by observing the length of time in days taken for the scar to drop off, with no trace of raw wound [23].

$$C (\%) = (H/A) 100 \dots\dots\dots (3)$$

Where H is the healed area and A is initial area of wound

Histopathological analysis of the wound

To prevent damage to the center of the wound, skin samples were taken from the edge of the wound at 1, 3, 7, and 14 days after the wound was created in order to track the healing process. The tissue was preserved by immersing it in a 10 % neutral buffered formalin solution for 24 hours. The tissue was then parched by traversing a couple of alcohol solutions (85, 90 and absolute alcohol) and cleared of alcohol using xylene, and then infiltrated with melted wax of paraffin wax to provide a base for fragmenting. Thin slices 4 μ m thick were cut and mounted on glass slides, and they were smeared with eosin and hematoxylin to show the overall architecture of the tissue, with cytoplasm-stained pink with eosin and cell nuclei stained blue with hematoxylin. Histopathological parameters were assessed qualitatively by observing the stained sections under a microscope [24].

Statistical analysis

The SPSS statistical software, version 22.0 (IBM, USA), was used to analyze the data. Data were displayed as mean $\pm$ standard deviation (SD) and comparison was done using Student's t-test. Statistical significance was adjudged at $P < 0.05$.

RESULTS

Yield and physical properties

The powdered extract was dark green and had a yield of 15.57 % w/w. The acid-insoluble, water-soluble and total ash contents of the dried leaves were found to be 1.73, 3.23 and 5.875 % w/w, respectively.

Phytochemical analysis

The result of the phytochemical analysis is presented in Table 3.

Particle size analysis

Particle size analysis, as presented in Figure 1 below, revealed that majority of the particles fell within nanoparticle size with mean size of 73.35

nm and polydispersity index (PDI) of 0.404 suggesting that particle size distribution is averagely good and not a heterogeneous system.

Table 3: Phytochemical analysis of *Annona muricata* leaf methanol extract

Phytochemical constituent	Results
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Terpenoids	+
Reducing sugars	+
Cardiac glycoside	+

Key: + (present), - (absent)

Viscosity studies

The viscosity of the gel formulations is presented in Figure 2. At a shear rate of 6 rpm, the viscosity ranged from 21859 to 99745 mPa s, with formulation F1 being the most viscous and formulation F5 the least viscous. Viscosity showed an inverse proportional relationship with shear rate.

Physicochemical properties of gel formulations

The physicochemical parameters of the formulated gels are presented in Table 3 below. Values of the spreadability ranged from 5.5 ± 0.05 to 9.7 ± 0.07 cm, indicating that the gel formulations were easily spreadable.

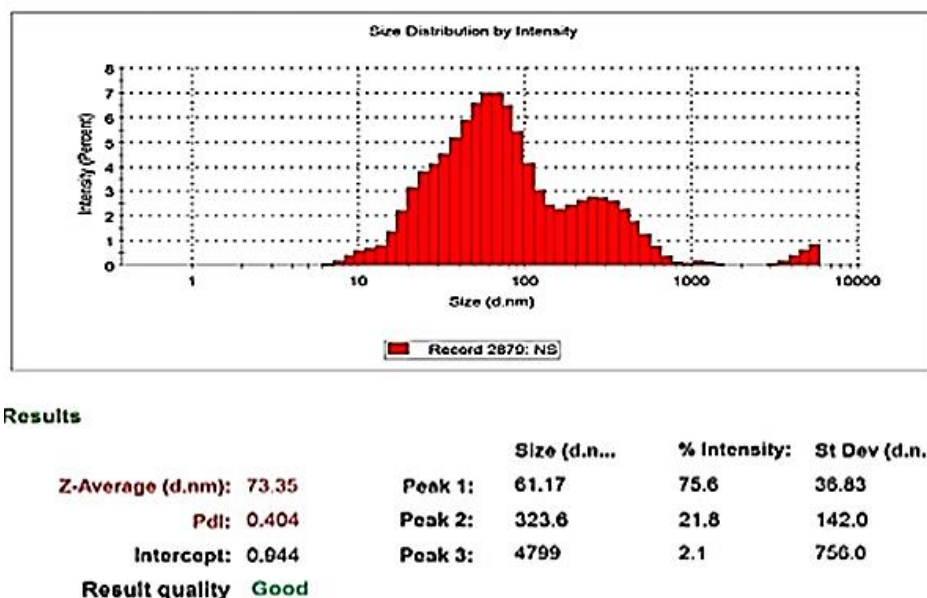


Figure 1: Particle size analysis of AME-nanosuspension

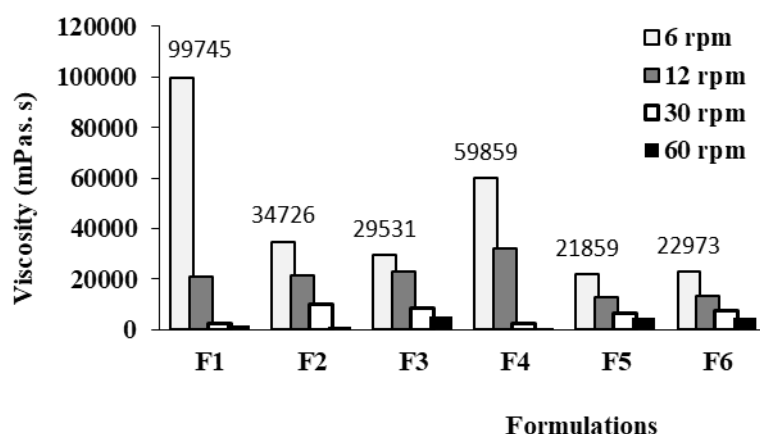


Figure 2: Viscosity chart for *Annona muricata* nanogels. F1 = 1% AME-nanogel; F2 = 2% AME-nanogel, F3 = 3% AME-nanogel; F4 = 1% AME-crude gel; F5 = 2% AME-crude gel, F6 = 3% AME-crude gel

The control formulation F7 (blank gel) had the least spreadability value of 5.5 ± 0.05 cm. The pH values of all formulations were within the range of 6.8 - 7.0, which falls within the tolerable pH range for topical preparations [25]. There was no skin irritation observed for all the formulations, even after 72 hours of the study, indicating that the gel formulations were safe for use.

Anti-inflammatory activities

The anti-inflammatory effect of *Annona muricata* was concentration-dependent, comparable to previous studies [26], and a faster onset of action was observed in the nanoformulation than in the crude extract. From the result presented in Figure 3 below at the third hour, formulation F3 (3 % AME-nanogel) achieved significant ($p < 0.05$) percentage paw size inhibition of 83.33% compared to the control (blank gel) and standard (methylsalicylate) of 17.65 and 50 %, respectively. At the fifth hour, F3 (3 % AME-nanogel) achieved 100 % paw size inhibition as against 58.82 and 96.43 % for control and standard, respectively.

Wound healing studies

Formulation F3 (3 % AME-nanogel) compared favourably with the standard (3 % Gentamicin) and gave a significant ($p < 0.05$) percentage wound closure of 94.87 % on day 15, as against 64.35 % for control (blank gel) and 100 % for the standard (3 % Gentamicin). This is presented in Table 4 below.

Histological evaluation of wound tissues

Histologies of excised wound tissues are presented in Figure 5 (a-d) below. The 3 % AME nanogel (F3) treated group showed angiogenesis and fibroblastic activity with evidence of substantial collagen deposition in wounds. There was restoration of adnexa and substantial re-epithelialization, indicative of wound healing (Figure 5 a). In 3 % AME crude gel (F6) treated wound tissues, there was evidence of fibroblast proliferation and collagen synthesis indicative of re-epithelialization at wound edges, but not much re-epithelialization in center of the wounds (Figure 5 b).

Table 3: Physicochemical properties of gel formulations

Formulation	pH	Spreadability (cm)	Skin irritation
F1	6.80 ± 0.04	7.2 ± 0.08	No reaction
F2	6.90 ± 0.05	9.5 ± 0.06	No reaction
F3	6.98 ± 0.02	9.7 ± 0.07	No reaction
F4	6.82 ± 0.04	6.7 ± 0.06	No reaction
F5	6.88 ± 0.05	7.7 ± 0.04	No reaction
F6	6.96 ± 0.04	8.2 ± 0.05	No reaction
F7	7.00 ± 0.01	5.5 ± 0.05	No reaction

F1 = 1 % AME-nanogel; F2 = 2 % AME-nanogel, F3 = 3 % AME-nanogel; F4 = 1 % AME-crude gel; F5 = 2 % AME-crude gel, F6 = 3 % AME-crude gel, F7 = blank gel

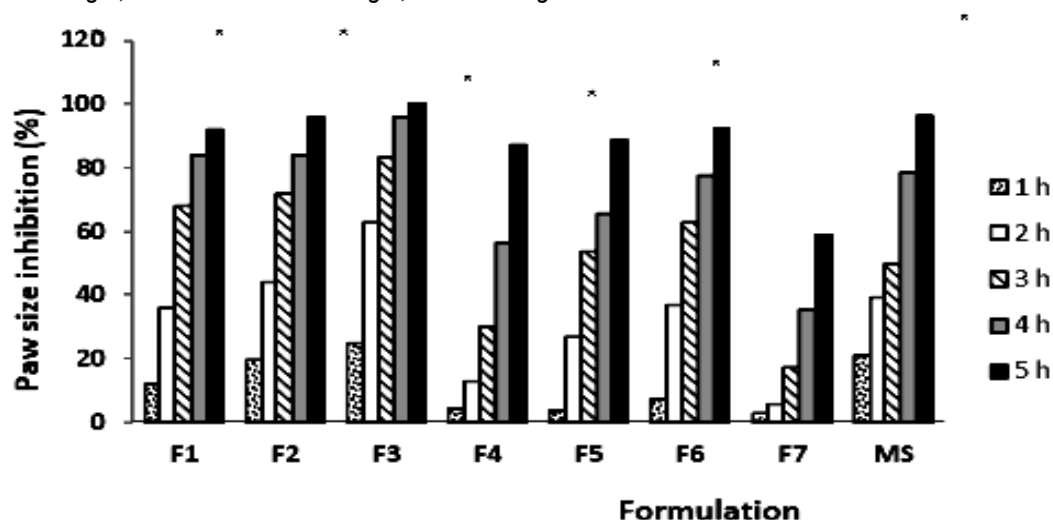


Figure 3: Percentage inhibition of paw edema size for AME-nanogels and AME-crude gels compared to control and methylsalicylate-treated groups (* $P < 0.05$, significantly different from control group). **Key:** F1 = 1% AME-nanogel; F2 = 2% AME-nanogel, F3 = 3% AME-nanogel; F4 = 1% AME-crude gel; F5 = 2% AME-crude gel, F6 = 3% AME-crude gel, F7 = blank gel; MS = 1% methyl salicylate gel

Table 4: Percentage wound contraction

Formulation	Wound contraction (%)						
	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15
F1	8.50 ^a	17.50 ^a	37.50 ^a	56.00 ^a	67.50 ^a	74.00 ^a	82.00 ^a
F2	14.67 ^b	28.00 ^b	42.22 ^a	61.33 ^b	72.00 ^a	83.11 ^b	88.89 ^b
F3	27.18 ^c	43.59 ^c	51.28 ^b	61.54 ^a	71.79 ^a	87.18 ^b	94.87 ^c
F4	2.17 ^d	13.04 ^a	25.22 ^c	37.39 ^c	46.96 ^b	64.35 ^c	73.91 ^d
F5	8.57 ^a	17.62 ^a	29.53 ^c	40.95 ^c	51.43 ^b	69.05 ^c	82.86 ^a
F6	10.00 ^a	19.09 ^d	36.36 ^a	58.18 ^a	68.18 ^a	79.55 ^a	90.00 ^b
F7	0.87 ^d	6.09 ^e	18.26 ^d	29.13 ^d	37.39 ^c	50.00 ^d	64.35 ^e
GC	23.08 ^e	34.35 ^f	51.79 ^b	68.21 ^b	79.49 ^d	88.21 ^b	100.00 ^f

Values are mean percentage wound contraction; different letters within a column indicate significant differences ($p < 0.05$). **Key:** F1 = 1% AME-nanogel; F2 = 2% AME-nanogel; F3 = 3% AME-nanogel; F4 = 1% AME-crude gel; F5 = 2% AME-crude gel; F6 = 3% AME-crude gel; F7 = blank gel; GC = 1% Gentamycin ointment

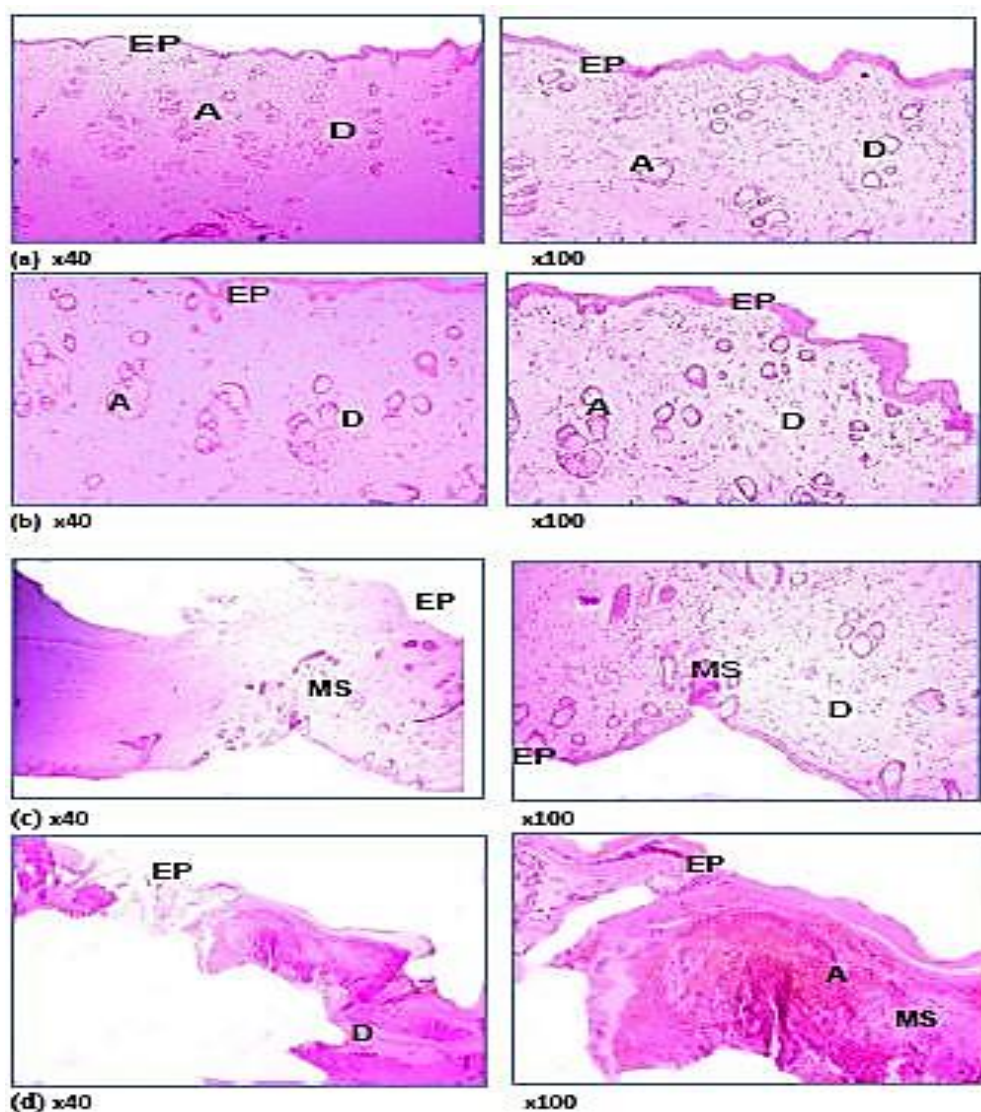


Figure 5: Histopathology of wound skin tissues at day 20. (a) Animals treated with 3% AME-Nanogel, (b) Animals treated with 3% AME-Crude gel, (c) Animals treated with standard 3% gentamicin ointment. (d) Control animal treated with blank gel. (EP: Epidermis, A: Adnexia, D: Dermis, MS: Myxoid Stroma)

The 3 % Gentamicin ointment-treated group showed restored skin structures with well-shaped, almost normal epidermis, remodeling of adnexa, and substantial fibrosis, re-epithelialization and collagen tissue within the dermis (Figure 5 c). In the control group (Blank gel), wound tissues showed persistent inflammation and little proliferation at wound edges, which indicated poor wound-healing rate. In addition, features showed an incision area (IA) with loss of epidermal projection into the dermis, indicating that complete wound closure did not take place (Figure 5 d).

Image of wound

Figure 6 below is the pictorial view of a representative group treated with the optimized formulation F3 (3 % AME-nanogel) showing the healed skin tissue after 20 days of treatment.



Figure 6: Images showing improvement of excision wound tissue in the optimized formulation F3 (3 % *Annona muricata* nanogel) treated group

In drug delivery, a PDI of 0.3 and below is adjudged acceptable and indicates a homogenous population [27]. The polydispersity index in this study was 0.404 and this shows that the size distribution of particles was fair.

The formulations displayed shear thinning behaviour because their viscosity decreased with increasing rates of shear (6, 12, 30 and 60 rpm). This pseudoplastic behaviour is of great advantage because high spreadability occurs during application to skin, which controls drug permeation through the skin as well as retention at the site of application when not sheared. The effectiveness of topical anti-inflammatory and wound healing preparations depends on this [28].

The repression of carrageenan-induced paw inflammation after the third hour is congruent with therapeutic doses of most clinically effective anti-inflammatory agents [29]. From the result

Complete wound healing had taken place; skin is back to normal with complete growth of fur over the area pending.

DISCUSSION

The particle size analysis of AME-nanogel reveals that majority of the particles fell within the nanosize range (1 -1000 nm). The polydispersity index (PDI) mean particle size of the nanoformulation was 0.404 and 73.35 nm, respectively, indicating fair particle size distribution and nanoparticle size range. The numerical value of PDI ranges from 0.0 (for an absolutely uniform monodisperse sample about the particle size) to 1.0 (for a highly polydisperse sample with numerous particle size populations).

presented in Figure 3 above at the third hour, formulation F3 (3 % AME-nanogel) achieved significant ($p < 0.05$) percentage paw size inhibition of 83.33 % compared to the control and standard of 17.65 and 50, respectively. Formulation F3 (3 % AME-nanogel) gave the highest anti-inflammatory activity with 100 % edema inhibition after 5 hours. The standard, methyl salicylate, had a slower onset of action than the nanogels, but at 5 hours achieved 96.43 % paw size inhibition. Previous studies also showed a similar anti-inflammatory profile of the leaf extract of the *Annona muricata* plant [30]. Several mediators, including histamine, 5-hydroxytryptamine (5-HT), bradykinin, and prostaglandins (PGs), work in synergy to cause this inflammatory reaction in the rat hind paw carrageenan-induced edema [31]. The anti-inflammatory effect of *A. muricata* through reduction of proinflammatory release and inhibition of cyclooxygenase (COX) activity is a

possible mechanism for inhibition of carrageenan-induced edema mediated by bioactive compounds in the alkaloid and terpenes components of the extract [32].

Annona muricata leaf methanol extract administered topically, in the form of a gel, revealed a healing effect on the excision wound in the experimental model studied. This result is in line with previous findings [33]. Comparing the wound closure profile of corresponding concentrations of the AME-nanogel and AME-crude gel, it can be deduced that the nanoformulation improved the wound healing profile of *Annona muricata* crude methanol extract. The optimized formulation F3 (3 % AME-nanogel) compared favourably with the standard and gave a significant ($p < 0.05$) percentage wound closure of 94.87 % on day 15, as against 64.32 % for blank gel. Enhanced activity of the nanogel could be due to increased bioavailability and solubility of the extract by decreasing particle size to an average size of 73.35 nm, which is similar to the findings of some researchers [34]. The remarkable wound closure observed after topical application of *Annona muricata* nanogel F3 (3 % AME-nanogel) in comparison with the control (blank gel, F7), was corroborated histologically by the early appearance of granulation tissue, rapid re-epithelialization and a well restored dermis with its adnexa as seen in Figure 5 a (3 % AME-nanogel treated group) and Figure 5 d (control treated group).

CONCLUSION

The findings suggest that *Annona muricata* methanol leaf extract possesses notable anti-inflammatory and wound healing properties, and the formulation of the extract into nanosuspension enhanced the activity significantly, indicating its potential topical use for the treatment of wounds. This also corroborates and justifies the traditional use of *Annona muricata* leaves for the remedy of wounds, as claimed in folklore literature. However, more investigations involving computational studies, in-silico modeling, gas chromatography–mass spectrometry (GC-MS) are required for possible identification of specific bio-active compounds and receptor interaction.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was carried out by the author(s) named in this article, and all liabilities relating to claims to the content of this article will be borne by the authors.

Ethical approval

Approval was granted by the Research and Ethics Committee of the Faculty of Basic Medical Sciences of the Delta State University, Abraka (approval no. REC/FBMS/DELSU/22/155).

Availability data and materials

The data supporting the research findings are available from the corresponding author upon request.

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