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EVALUATION OF THE WOUND HEALING EFFECT OF THE SEED POD OF *PENTACLETHRA MACROPHYLLA* BENTH IN DIABETIC FOOT ULCER

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Abstract
Introduction: *Pentaclethra macrophylla*, commonly referred to as the Oil Bean tree, is an indigenous plant in tropical Africa with extensive historical application in traditional medicine for addressing various health issues. Notably, the seed pod, considered a byproduct, has received scant attention despite studies highlighting the wound healing attributes of the seed. Limited research has been conducted on the seed pods of *Pentaclethra macrophylla*. This study aimed to assess the wound healing efficacy of *Pentaclethra macrophylla* seed pod extracts on diabetic foot ulcers.
Methods: The seed pod was pulverized, the sample extracted using a mixture of two solvents: dichloromethane and methanol in a 1:1 ratio, followed by drying on a rotary evaporator. Phytochemical analysis was carried out using standard procedures, GC-MS analysis was carried out, and the extract was incorporated into cream formulations with different concentrations. The cream was topically administered to the rats to evaluate wound healing potential using an excision wound model over a 21-day period, while dermazin served as a positive control in the excision wound model.
Results: The phytochemical analysis revealed the presence of tannins and triterpenoid in the extract. The GC-MS analysis identified five bioactive compounds in the extract. The wound-healing assay results showed that the animal groups treated with the extracts exhibited notable wound-healing properties compared with the positive control group. In particular, the 4% n-Hexane, 4% methanol, and 2.5% methanol fractions along with glibenclamide demonstrated superior wound healing activity compared with the positive control group at $P<0.05$. This outcome may be attributed to the presence of phytoconstituents and the identified bioactive compounds.
Conclusion: The wound healing activities investigated showed that both the polar and non-polar extracts of the seed pods of *P. macrophylla* had very potent wound healing activities. These results provide a basis for further studies on the therapeutic potential of this plant in wound healing management.

1.0 INTRODUCTION

A diabetic ulcer is a chronic wound that commonly occurs in individuals with diabetes, a metabolic disorder characterized by elevated blood sugar levels. The development of these ulcers is often linked to a combination of

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factors associated with diabetes, including peripheral neuropathy, poor circulation, and compromised immune function (Abdissa, 2020).

Peripheral Neuropathy: Diabetes can lead to nerve damage, particularly in the extremities, a condition known as peripheral neuropathy (Mold et al., 2004). This results in reduced sensation, making it difficult for individuals to detect injuries, pressure points, or friction on their feet. Consequently, minor wounds or pressure areas may go unnoticed and untreated, evolving into ulcers (Armstrong et al., 2017).

Poor circulation, especially in the lower limbs. Reduced blood flow hinders the delivery of oxygen and nutrients to tissues, impairing the body's natural ability to heal. This contributes to the persistence of wounds and increases the risk of infection.

Compromised Immune Function: Diabetes can weaken the immune system, making individuals more susceptible to infections. Once an ulcer forms, the body's ability to fight off bacteria is compromised, heightening the risk of localized or systemic infection (Zhang et al., 2003).

Location on the Feet: Diabetic ulcers commonly manifest on the feet, particularly the soles, because of the constant pressure and friction associated with walking. The lack of protective sensation and diminished blood supply in the feet further intensify the risk of ulcer formation in these areas (Shiffman and Low, 2020).

Delayed Healing: Diabetic ulcers tend to heal slowly, and prolonged open wounds increase the likelihood of complications. If not properly managed, these ulcers can deepen, affect deeper tissues, and may even lead to the development of more serious conditions such as cellulitis, osteomyelitis (bone infection), or, in severe cases, amputation (Hannan and Attinger, 2009).

Proper management of diabetic ulcers involves a multidisciplinary approach, including meticulous foot care, wound dressings, infection control, and lifestyle modifications. Regular monitoring and medical intervention are crucial to prevent complications and promote healing in individuals with diabetes (Kleopatra Alexiadou and Doupis, 2012). The tree of *Pentaclethra macrophylla*, also known as African oil beans, is a huge shrub with long bi-pinnate compound leaves that are endemic to West and Central Africa. It belongs to the Fabaceae family.

MATERIALS AND METHODS

3.1 COLLECTION OF THE PLANT MATERIAL

The seed pod of the *Pentaclethra macrophylla* plant was collected from a bio-reserve in Ethiope West LGA of Delta State Nigeria in February 2023. Soon after collection, the seed pods were cleaned and shade-dried. The plant was identified, confirmed, and authenticated by Dr. Suleiman, a Taxonomist in the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt Rivers State, Nigeria. (Voucher No: UPHF0598). A voucher specimen of the plant was deposited in the Departmental herbarium for future reference. After drying, they were crushed to a powder and stored in an airtight plastic container for further use.

3.2 EXTRACTION

The pulverized powder (500 g) was extracted with a 1:1 mixture of dichloromethane and methanol by continuous extraction in a Soxhlet extractor. The filtrate was concentrated using a rotary evaporator to obtain the dichloromethane-methanol extract of *Pentaclethra macrophylla*. The total plant sample was partitioned into polar and non-polar fractions using n-hexane and 90% aqueous methanol. The obtained crude extract was weighed and stored at 4°C for further analysis.

3.3 PHYTOCHEMICAL ANALYSIS

The plant extract was subjected to preliminary analytical studies using the methods described by Trease and Evans (2009) to determine the various types of phytochemicals and compounds present.

3.4 GC-MS ANALYSIS OF THE DICHLOROMETHANE-METHANOL EXTRACT OF *PENTACLETHRA MACROPHYLLA* SEED POD

Using an Agilent 7890B GC system in conjunction with an Agilent 5977A MSD and a Zebron-5MS column (ZB-5MS 30 m × 0.25 mm × 0.025 μm) (5%-phenylmethylpolysiloxane), gas chromatography–mass spectrometry (GC-MS) analysis was quantitatively performed. The carrier gas was GC-grade helium flowing at a steady 2 mL/min rate. Before use, the total extract was filtered and dissolved in ethanol. An ultimate temperature of 300°C was attained by progressively increasing the column temperature from 60°C to 10°C each minute. The GC-MS analysis took 40 min to complete. Using a computer to compare the mass spectra with the NIST 11 MS library (National Institute of Standards and Technology library), the chemicals were identified.

FORMULATION OF THE CREAM

Formula for cream formation:

Plant Extract:	4%, 2.5%, and 1%
Emulsifying ointment:	30%
Shear butter	20%
Purified water to	100%

A 50 g preparation was made.

Preparation of the emulsifying ointment:

Formula from pharmaceutical Codex pc	79

Rx

Emulsifying wax (300 g)

White soft paraffin (500 g)

Liquid paraffin (200 g)

The formula was reduced by 50%

METHOD:

The three ingredients were accurately weighed and/or measured and transferred into a glass container. Subsequently, they were melted together in a water bath and stirred until cooled to form the emulsifying ointment.

Base preparation:

Emulsifying ointment (15 g) and shear butter (10 g) were combined in a beaker and gently heated over a water bath until melted. 23 ml of purified water was measured into another beaker and placed over the water bath. A thermometer was used to regulate the temperature to 60 °C. The mixture was transferred into a porcelain mortar and stirred. The base was transferred into a wide-mouthed plastic container, capped, and labeled appropriately

Method of cream formation:

Emulsifying ointment (15 g) and shear butter (10 g) were combined in a beaker and gently heated over a water bath until melted. 23 ml of purified water was measured into another beaker and placed over the water bath. A thermometer was used to regulate the temperature to 60 °C. The mixture was transferred into a porcelain mortar and stirred; 23 g of Purified Water was added gradually while still stirring. Two grams of the plant extract was added and stirred together until a uniform and consistent cream was formed. The cream was transferred into a wide-mouthed plastic container, capped, and labeled appropriately.

For the 2.5% and 1% cream formulations, 1.25 g and 0.5 g of the plant extract sample were added, respectively, in place of 2 g. With the aid of a measuring cylinder, 23.75 ml and 24.5 ml of purified water was added respectively.

3.5 PHARMACOLOGICAL STUDY

3.5.1 ANIMALS AND MANAGEMENT

Wistar albino (male and female) rats (46) weighing between 150 g and 200 g were obtained from the Department of Experimental Pharmacology& Toxicology animal house, University of Port Harcourt. They were sorted and housed in standard cages with housing conditions of 12:12 light: dark cycles. They were fed standard rat pellets and water ad libitum. The rats were maintained at room temperature and allowed to acclimatize to the new environment for 2 weeks.

3.5.2 DRUG

Commercially available Dermazin was used as the control drug. It was applied topically over the wound area.

3.5.3 INDUCTION OF DIABETES

Diabetes was induced in rats using Alloxan Monohydrate (Sigma-Aldrich), a compound that has preferential toxicity toward pancreatic beta cells. Diabetic conditions were induced by a single intraperitoneal injection of Alloxan at a concentration of 150 mg/kg body weight in overnight fasted rats. After 3 days of induction, blood samples were collected from the tail and measured using an Accu-Check glucometer. Animals with fasting blood glucose levels of 10 mMol/L were considered diabetic.

3.5.4 EVALUATION OF THE WOUND HEALING EFFECT OF *PENTACLETHRA MACROPHYLLA* ON EXCISION OF WOUND

On the fourth day after the induction of Alloxan, the diabetic rats were randomly classified into 11 groups.

Group 1 (Diabetic Treated with 4% N-Hexane Fraction)

Group 2 (Diabetic Treated with 1% N-Hexane Fraction)

Group 3 (Diabetic Treated with 4% Methanol Fraction)

Group 4 (Diabetic Treated with 2.5% Methanol Fraction)

Group 5 (Diabetic Treated with 1% Methanol Fraction)

Group 6 [Diabetic-treated 4% Methanol Fraction + Glibenclamide (drug))

Controls

Group 7 (Diabetic Untreated)

Group 8 (Non Diabetic Non Treated)

Group 9 (Diabetic and untreated

Group 10 (Diabetic Treated with Dermazin)

Group 11 (Diabetic Treated with Base)

INDUCTION OF THE WOUND

On wounding day, the rats were anesthetized with diethyl ether before the creation of the wounds. The dorsal fur of the animal was shaved with an electric clipper, and the area of the wound to be created was outlined on the back of the animal. A 1.5-cm-wide excision wound of 1.5 cm in width was created along the markings using toothed forceps, a surgical blade, and pointed scissors. The entire wound was left open. All surgical procedures were performed under sterile conditions. The wound was left for several days until it turned into an ulcer. The cream was applied gently to cover the ulcerated area once daily for 21 days. The wound area and wound contraction were monitored.

3.6 ESTIMATION OF THE PARAMETERS

Measurement of Wound Contraction

The progression of wound healing was judged by periodic assessment of contraction of excision wounds. Wound contraction was monitored by tracing the outline of the wound on a tracing sheet and then using a graph sheet to

calculate the area of the wound size. All animals in each group were monitored until complete healing of wounds occurred, and the day at which each wound healed was recorded. The mean of all healed wounds was determined. It was calculated using $\frac{total\ area - healed\ area}{total\ area} \times 100$

3.7 Materials/Method for histology

The following materials and reagents were used for the histology study: formalin for tissue fixation, alcohol or ethanol for dehydration, xylene for clearing tissue, paraffin wax for impregnation and embedding, DPX mountant for keeping specimen in place and protecting from accidental contact, glycerin for making section adhesive, dyes for staining, processing cassettes, embedding mold, water bath, frosted slides, staining racks and dishes, cover slips, rotary microtome, electric hot plate, hot air oven, plastic sample bottles, dissecting set, digital camera, and light microscope.

The following step-by-step procedures constitute techniques for preparing histological tissue specimens as modified from the methods of Slaoui and Fiette (2011). These include: Tissue Sampling, Tissue Fixation, dehydration, clearing, impregnation, embedding, sectioning, staining, Mounting and placing coverslips, and photomicrography.

3.8 STATISTICAL ANALYSIS

Each test was performed in quadruplets. The values are expressed as mean ± standard error of the mean (SEM). One-way analysis (ANOVA) was used to determine the significant differences among all columns against the control, and a P value < 0.05 was considered significant. All statistical analysis was performed using Graph Pad Prism version 8.0 software.

3.0 RESULTS

3.1 PHYTOCHEMICAL ANALYSIS OF *PENTACLETHRA MACROPHYLLA*

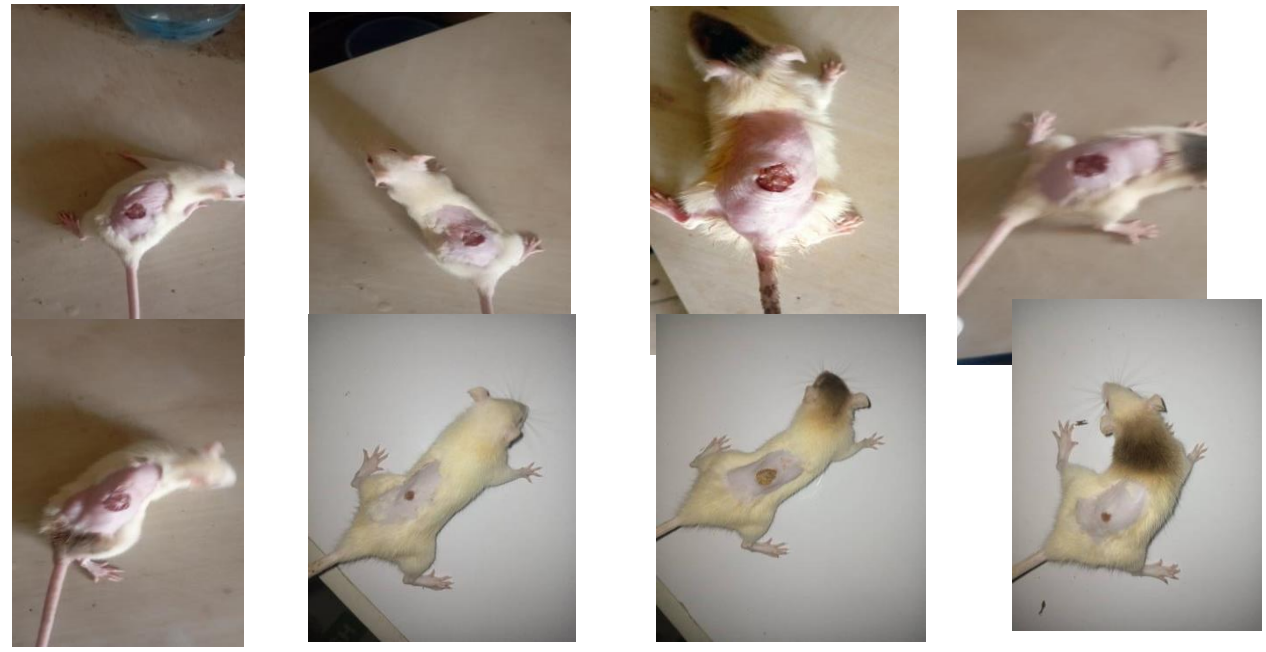
Following phytochemical screening of the total extract of *Pentaclethra macrophylla*, the extract contained various constituents such as Tannins, steroids, triterpenoid, and cardiac glycosides, whereas alkaloids, saponins, anthraquinones, and flavonoids were absent.

Table 1: Phytochemical constituents of the total extract of the seed pod of *Pentaclethra macrophylla.*

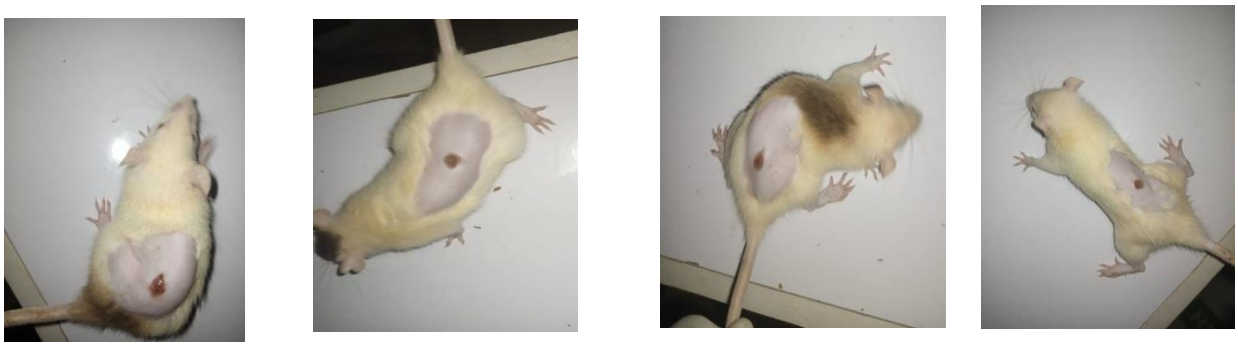
Constituent	Present/Absent
Alkaloids	Absent
Tannins	Present
Saponins	Absent
Flavonoids	Absent
Anthraquinones	Absent
Steroids	Present
Triterpenoids	Present
Cardiac glycosides	Present

4.2 PHARMACOLOGICAL STUDY RESULTS

After induction of the wound, the animals were left for some days untreated so that the wound would turn into an ulcer**.** Pictures of the wounds for different groups are presented in fig 4 below.



DAY 7



DAY 14

Fig 4: Picture of some rats and wound healing contraction. (Groups: base treated, Dermazin treated, 2.5% methanol fraction + Drug, 4% methanol fraction, 1% methanol fraction, 1% n-hexane fraction, 4% methanol fraction, diabetic untreated).

3.3: Fasting Blood Glucose Results

Alloxan was used to induce diabetes in the animals, and their blood glucose levels before and after induction were measured. The values are presented in table 2 below.

Table 2: Table showing the blood sugar readings for the various groups throughout the course of the experiment showing the diabetic or non-diabetic groups.

	before induction	after induction	7th day	14th day
Group 1	4.7 ± 0.4	11.2 ± 0.7	11.5 ± 0.7	12.9 ± 1.1
Group 2	4.4 ± 0.4	11.3 ± 0.6	11.2 ± 1.0	11.8 ± 1.6
Group 3	4.8 ± 0.5	11.9 ± 0.6	11.3 ± 0.7	15.3 ± 1.4
Group 4	4.5 ± 0.2	11.2 ± 0.9	11.4 ± 0.8	14.0 ± 1.6
Group 5	4.1 ± 0.5	11.2 ± 0.3	11.6 ± 0.9	15.1 ± 2.3
Group 6	4.0 ± 0.5	11.4 ± 0.6	12.0 ± 0.8	14.9 ± 1.7

3.4 Analysis of the Results

Table 3: The table below shows the mean and standard error of the mean of the control group and different concentrations of the plant extracts over a 21-day treatment of the wounds.

	Day 0	Day 7	Day 14	Day 21
Base	1.5±0	1.225±0.025	0.825±0.025	0.25±0.029
Dermazin	1.5±0	1.233±0.033	0.767±0.033	0.333±0.033
NONDIABETIC AND UNTREATED	1.5±0	1.15±0.029	0.875±0.025	0.35±0.029
DIABETIC AND UNTREATED	1.5±0	1.4±0	1.25±0.05	0.85±0.05
DIABETIC AND TREATED WITH DERMAZIN	1.5±0	1.067±0.033	0.533±0.033	0.033±0.033
1% METHANOL FRACTION	1.5±0	1.25±0.029	0.85±0.029	0.35±0.029
2.5% METHANOL FRACTION	1.5±0	1.225±0.025	0.85±0.029	0.325±0.025
4% METHANOL FRACTION	1.5±0	1.075±0.048	0.65±0.029	0.15±0.029
2.5% METH + DRUG	1.5±0	0.967±0.033	0.4±0.058	
1% N-HEXANE FRACTION	1.5±0	1.225±0.025	0.85±0.029	0.3333±0.033
4% N-HEXANE FRACTION	1.5±0	1.15±0.029	0.6333±0.033	0.1333±0.033

The chart below shows how the control groups compare with the different concentrations of the plant extracts (methanol and n-hexane fractions).

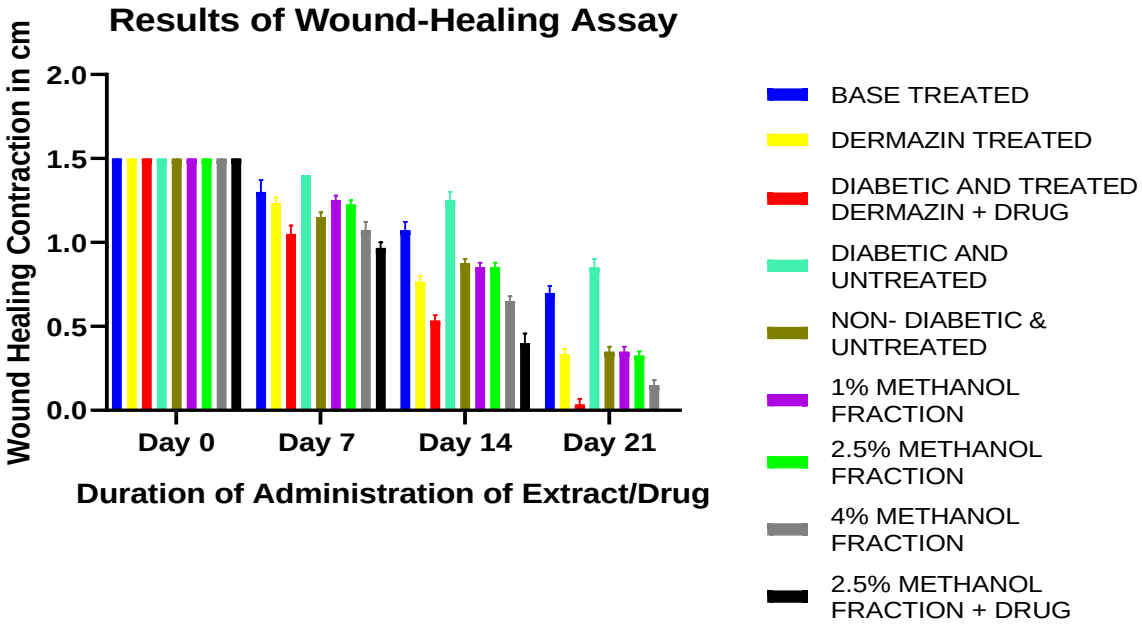


Fig 5: Chart comparing wound healing contraction control groups against methanol fractions

A comparison of the wound healing activities of the control groups and the n-Hexane fractions is presented in Figure 6.

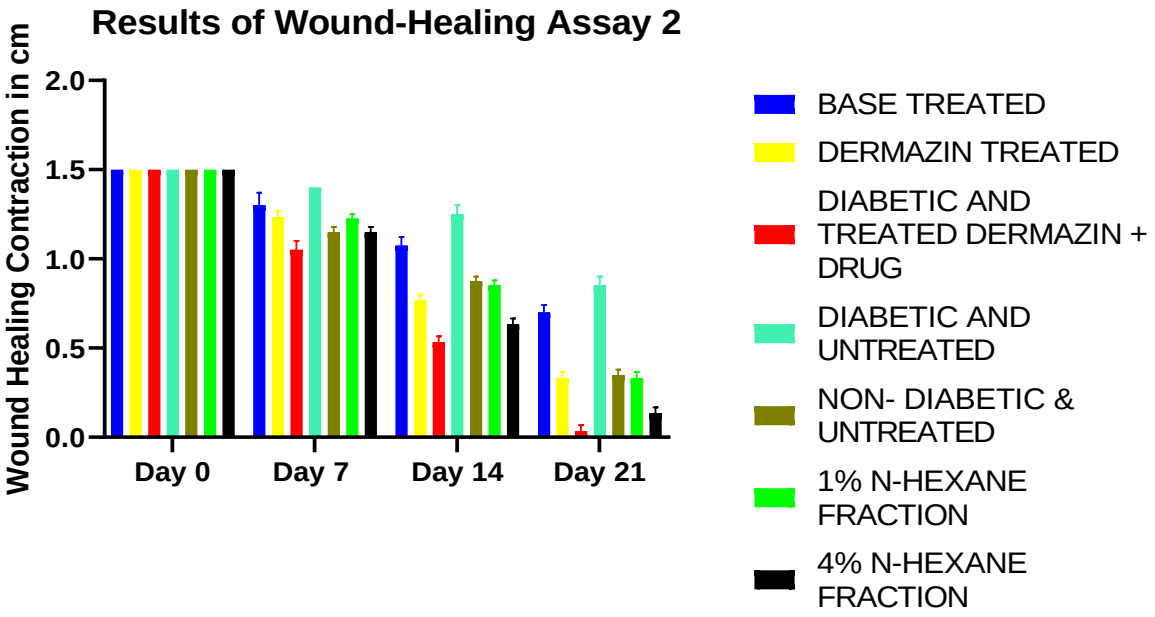


Fig 6: Chart comparing the wound healing contraction of the control groups against the n-hexane fraction

A comparison of the percentage wound healing contraction of the control groups against methanol and n-hexane fractions was made. These results are presented in figures 7 and 8 below.

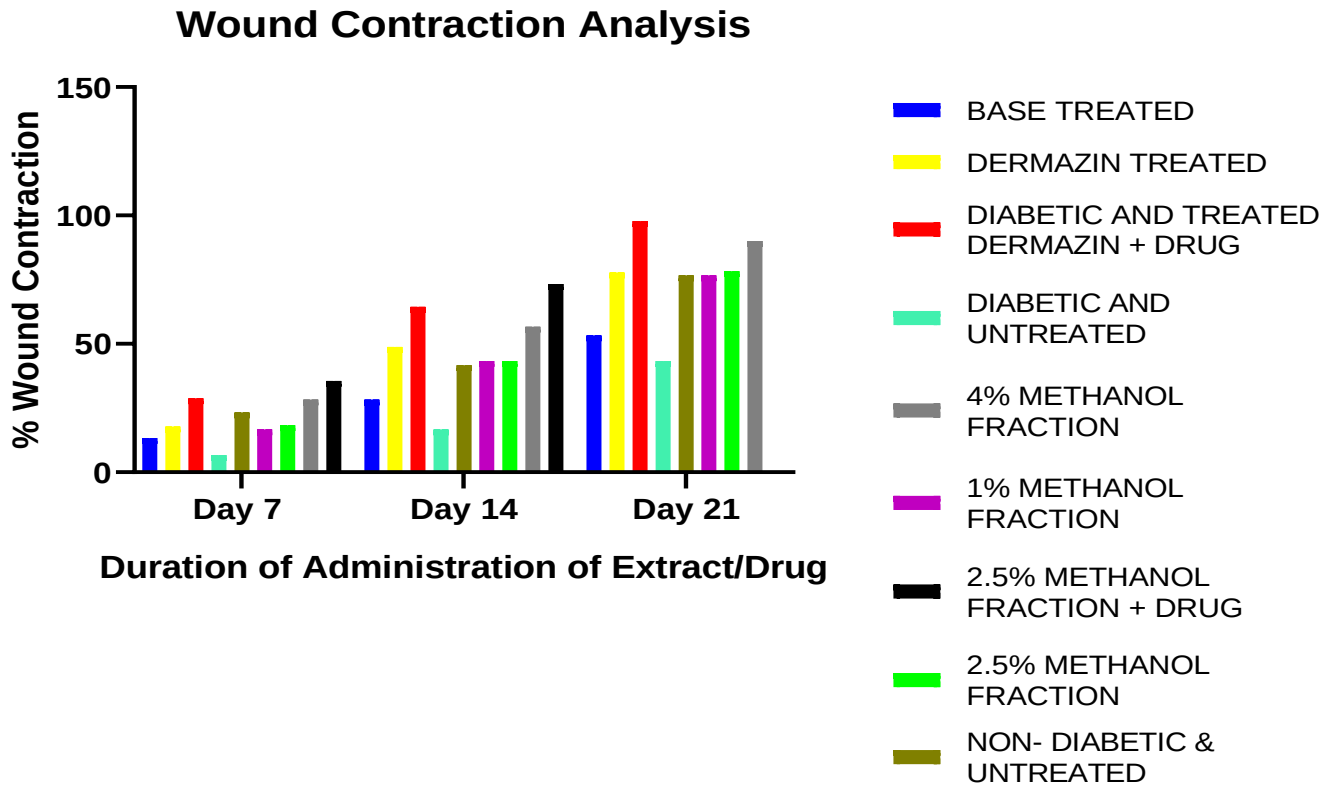


Fig 7: Chart comparing the percentage wound healing contraction of the control groups against the methanol fractions

Similarly, a comparison of the n-Hexane fractions and control groups was performed, as shown in Figure 8.

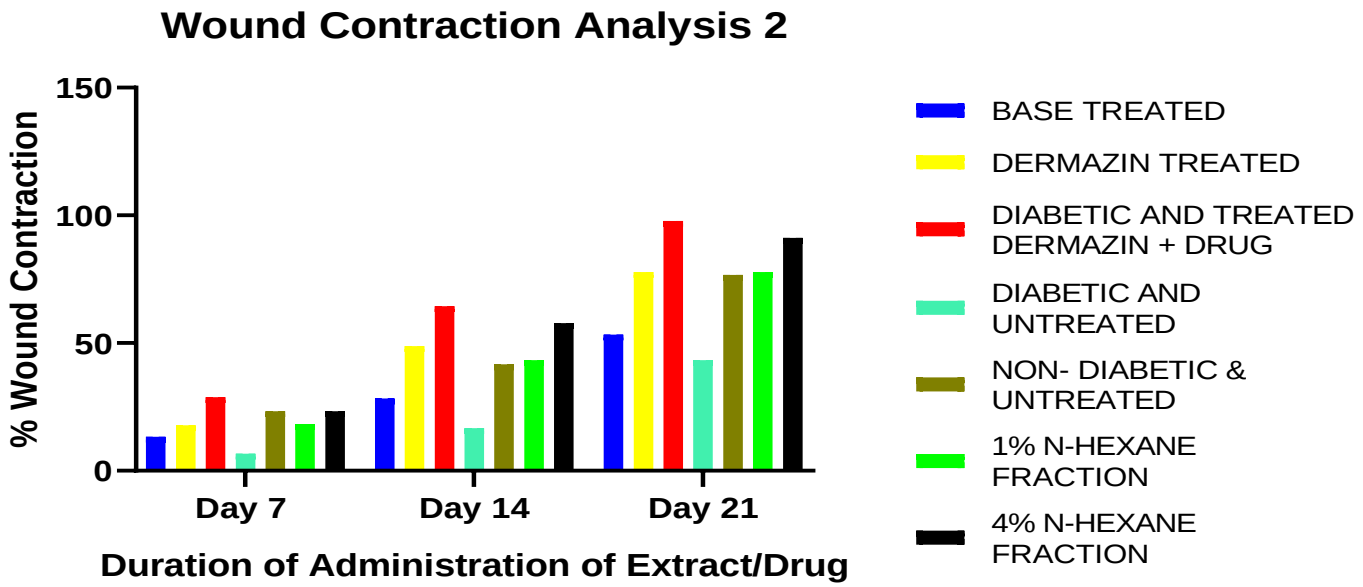
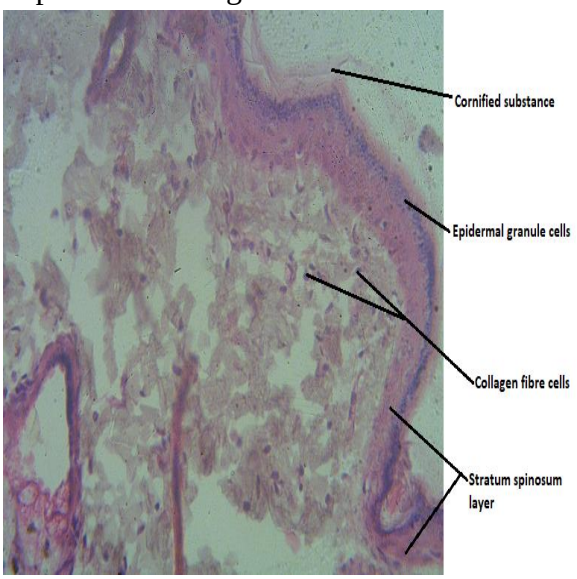


Fig 8: Chart comparing the percentage wound healing contraction of the control groups against the n-hexane fractions

4.5 Histological Results

The results of the histological investigations on the wound healing activities of the fractions and control groups are presented in Figure 9–18 below:



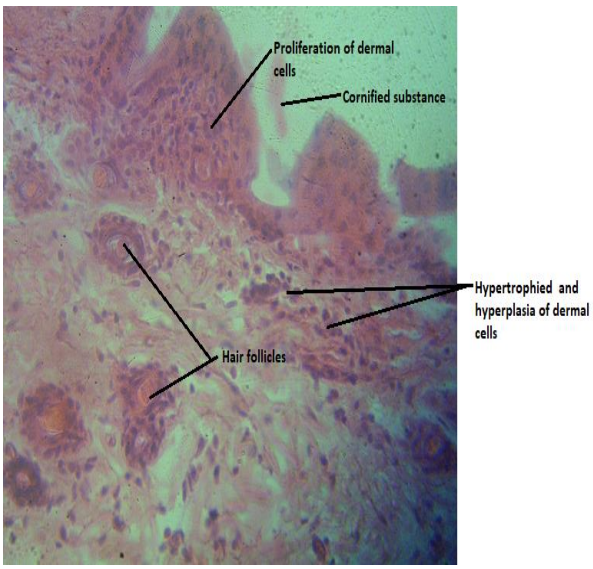


FIG. 9 (UNDIABETIC)
Photomicrograph of the rat skin showing cornified substance in the cornified layer, granule cells in the epidermis, and dermal cells in the stratum spinosum layer. Collagen fibers are also observed within the tissue. Tissue shows normal tissue appearance. H & E, X400

FIG. 10 (DIABETIC + DRUG)
Photomicrograph of rat skin tissue showing proliferation of dermal cells (with localized dermal cells hypertrophy and hyperplasia), hair follicles, and distinct collagen fibers and cells. Tissue shows a distinct microstructure. H & E, X400

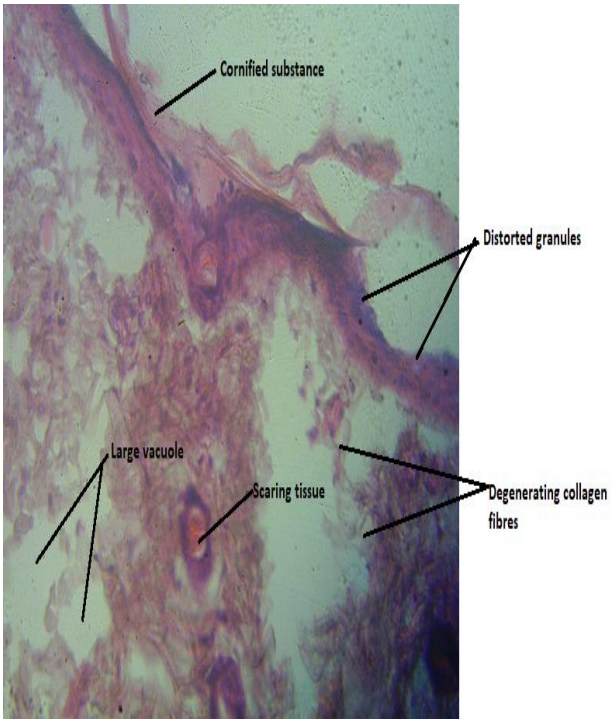


FIG. 11 (DIABETIC UNTREATED) Photomicrograph of rat skin tissue showing cornified layer with corn substance presence on granules of epidermal layer, distorted granules, and severe distortion of collagen fibers with large vacuoles. Tissue shows severe distortion of the microstructure. H & E, X400

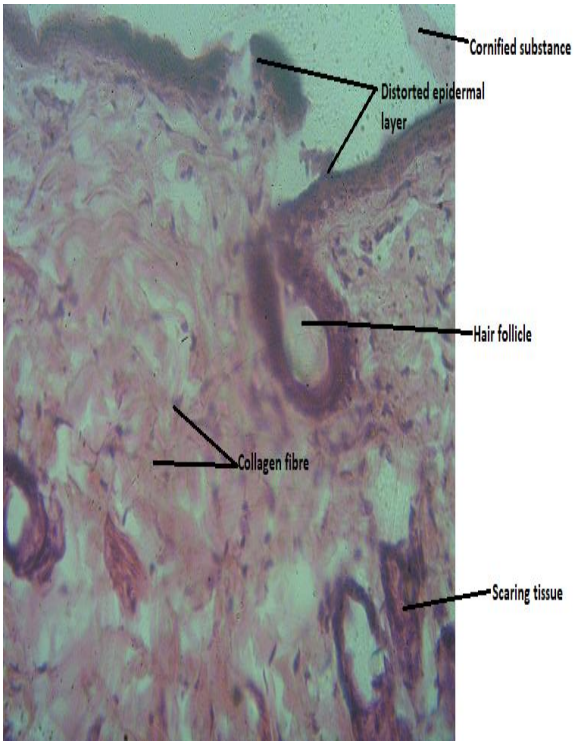
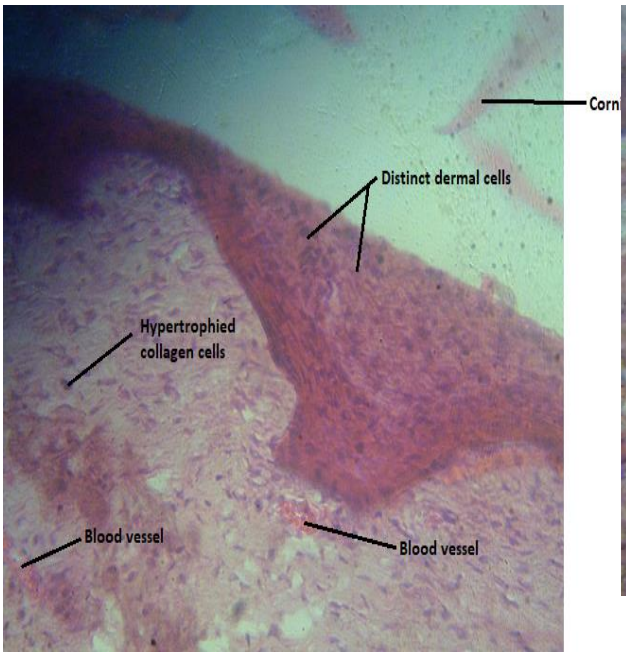


FIG. 12 (METHANOL 2.5% + DRUG) Photomicrograph of rat skin tissue showing distorted epidermal granules, scarring tissue, and distinct collagen fibers with distinct nuclei. H & E, X400



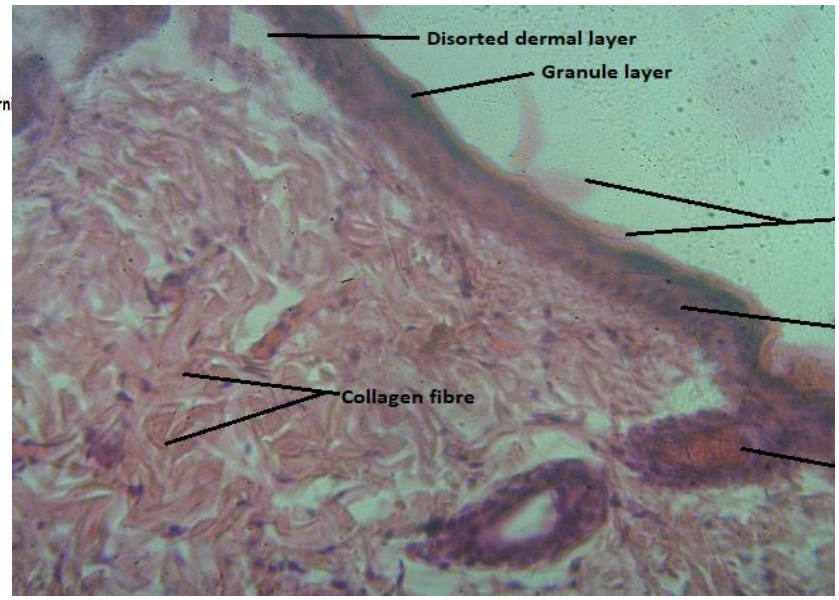


FIG. 13 (METHANOL 1% FRACTION)
Photomicrograph of rat skin tissue showing hypertrophy and hyperplasia in the presence of blood vessel deposits. Tissue shows severe distortion of the microstructure. H & E, X400

FIG. 14 (DERMAZIN TREATED)
Photomicrograph of rat skin tissue showing distorted dermal layer, cornified substance, distinct granules, and collagen fibers with distinct nuclei. The tissue shows mild distortion. H & E, X400

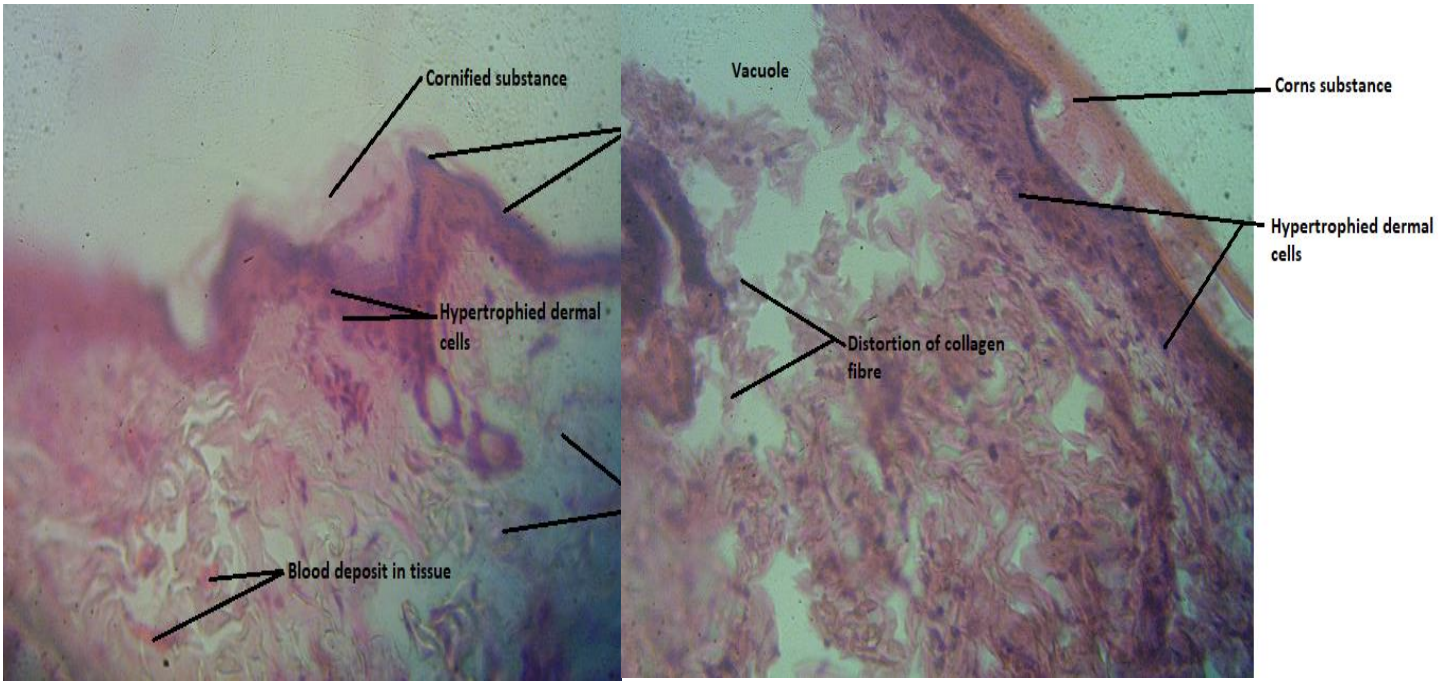


FIG. 15 (METHANOL FRACTION 4%)
Photomicrograph of rat skin tissue showing a cornified layer with corn substance present on granules of the epidermal layer, hypertrophied dermal cells, and distorted collagen fibers. Tissue shows mild distortion of the microstructure. H & E, X400

FIG. 16 (BASE TREATED)
Photomicrograph of the rat skin showing hypertrophied dermal cells, distorted collagen fibers and large vacuoles within the tissue. Distortion of the tissue microstructure is indicated. H & E, X400

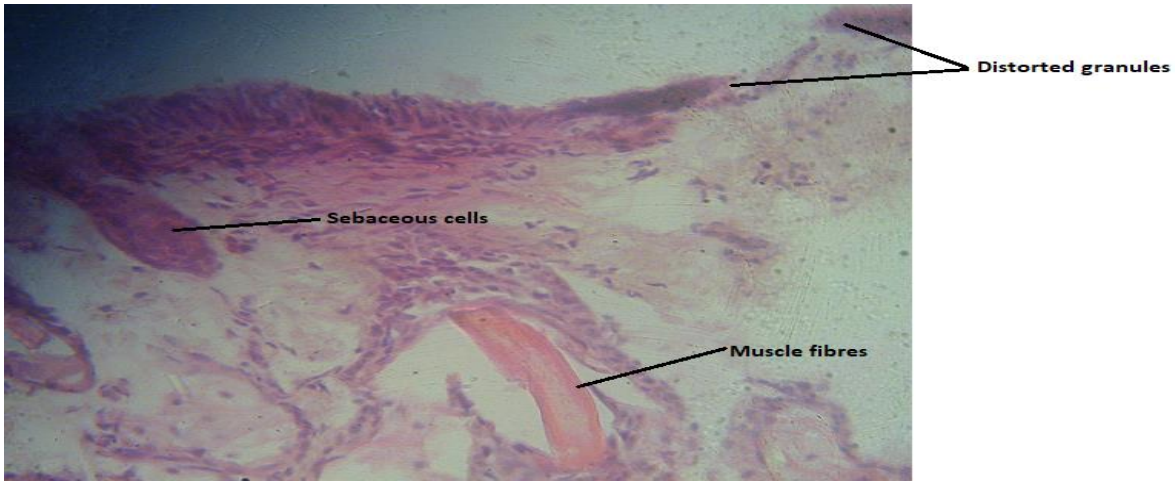


FIG. 17 (N-HEXANE FRACTION 4%)

Photomicrograph of rat skin tissue showing localized distortion of granules of the epidermal layer and distortion of collagen fibers with large vacuoles. Distinct muscle fibers and sebaceous gland cells were also observed. Tissue distortion of the microstructure is indicated. H & E, X400

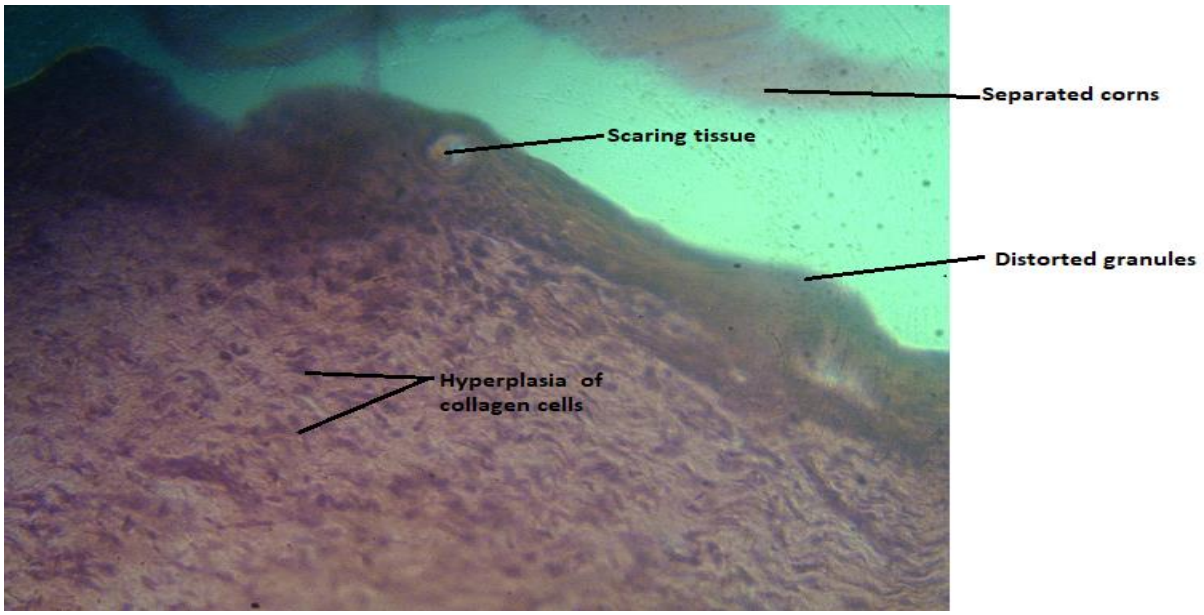


FIG 18 (METHANOL 2.5%): Photomicrograph of rat skin tissue showing hyperplasic dermal cells and mild distortion of granules in the epidermal layer. Tissue shows mild distortion of the microstructure. H & E, X400

The wound healing process entails a number of processes that involve the following phases: hemostasis phase, where coagulation occurs. This is caused by the necessity to form clots and enable the healing process to start. The inflammation phase is characterized by the release of neutrophils to destroy invading bacteria, whereas proliferation entails the formation of fibroblast cells, which aids the formation of collagen, leading to granulation, angiogenesis, and ultimately the replacement of the destroyed tissues. During the remodeling phase, granulation tissues are remodeled as dermal fibroblasts multiply. This results in the formation of scar tissues that are characteristically different from the original tissue.

4.6 GC-MS RESULTS

GC-MS screening was carried out on the n-hexane fraction of the plant extract, and tannins and triterpenoid were found to possess some compounds responsible for the wound healing properties of *Pentaclethra macrophylla.*

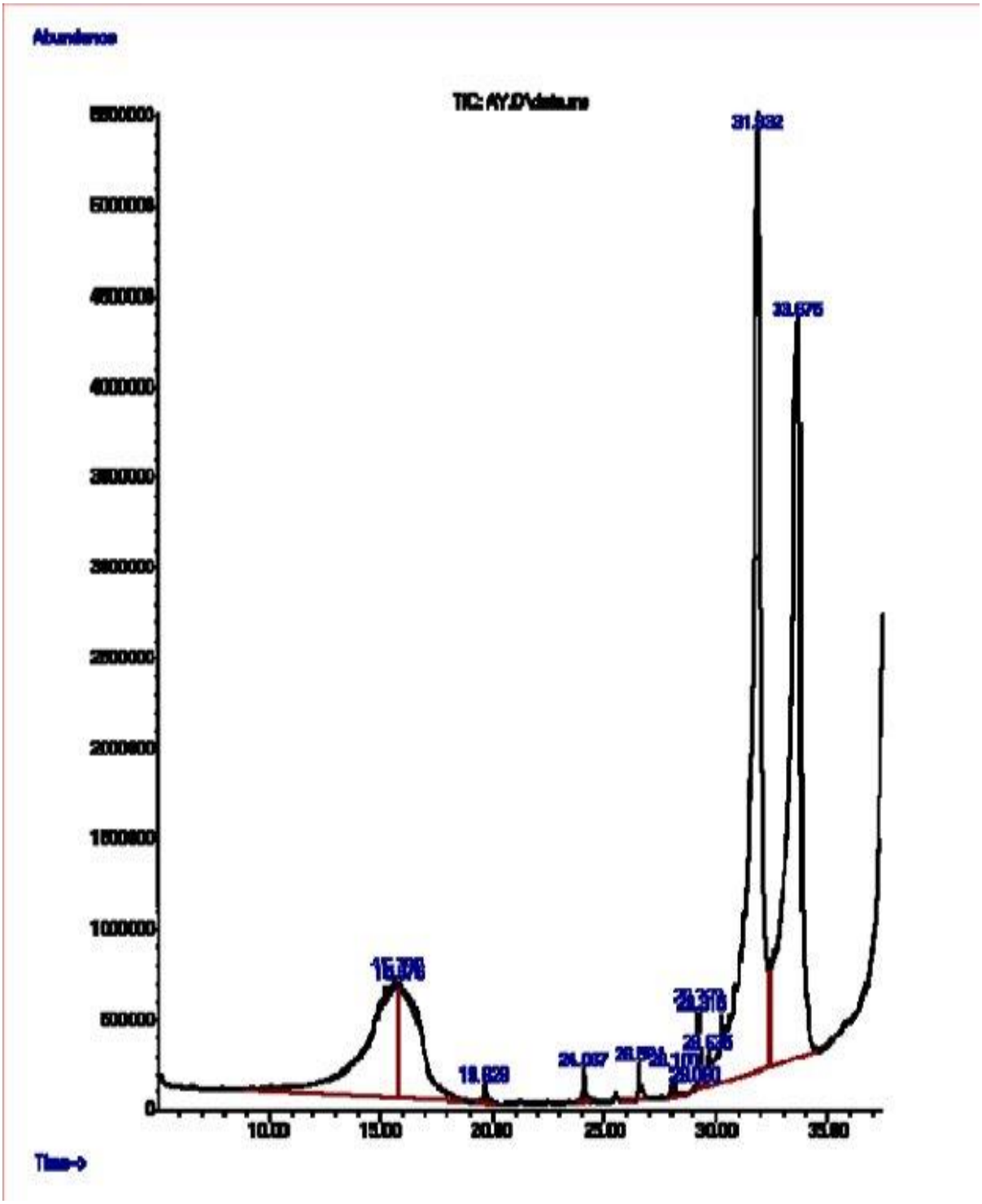


Fig 19: GC-MS chromatogram of the n-hexane fraction of the seed pod of *Pentaclethra macrophylla*.

The identified compounds whose match quality exceeded 90% are presented in Table 4.

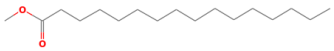
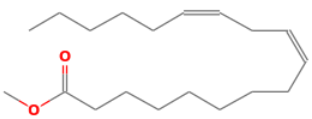
Table 4: Some compounds identified from the n-hexane extract of *Pentaclethra macrophylla*

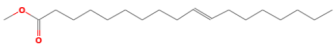
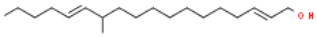
Retention	Quantity (%)	Compound

Time		
15.7901	0.6034	3,3,3-Trifluoro-N-(2-fluorophenyl)-2-(trifluoromethyl)propionamide
15.8758	9.0129	1H-Azepine, hexahydro-1-nitroso-
19.6295	0.1912	1-Tetradecene
24.0667	0.2944	E-7-Octadecene
26.5842	0.3385	Hexadecanoic acid methyl ester
28.1004	0.2076	1-Docosene
29.0896	0.0218	12-Methyl-E,E-2,13-octadecadien-1-ol
29.2202	0.4031	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
29.3151	0.5135	10-Octadecenoic acid methyl ester
29.6354	0.3883	Heptadecanoic acid, 16-methyl-, methyl ester
31.9324	40.879	12-Methyl-E,E-2,13-octadecadien-1-ol
33.6753	32.8875	9,12-Octadecadienoic acid (Z,Z)-

The pharmacologically active compounds are highlighted and shown in Table 5.

Table 5: Structural and pharmacological potentials of major bioactive compounds identified from the n-hexane extract of *Pentaclethra macrophylla*

Compound Name	Structure	Medicinal Uses
E-7-Octadecene		lubricant
Hexadecanoic acid methyl ester		antibacterial
9,12-Octadecadienoic acid (Z,Z)-, methyl ester		anti-inflammatory

10-Octadecenoic acid methyl ester		antibacterial, antidiabetic
12-Methyl-E,E-2,13-octadecadien-1-ol		anticancer and antioxidant

4.0 Discussion

Phytoconstituents are chemical compounds found in different plants that play important roles in such plants. *Pentaclethra macrophylla* has some phytochemical constituents such as tannins, triterpenoids, steroids, etc. These constituents are responsible for the acclaimed wound healing properties of the plant, but alkaloids, saponins, and flavonoids were absent from the plant.

Tannins, a class of natural polyphenolic compounds widely distributed in plants, possess astringent characteristics, binding to proteins, and causing tissue tightening and protein precipitation (De Melo et al., 2023). With recognized biological activities including antioxidant, anti-inflammatory, antimicrobial, and wound healing properties (Üstüner et al., 2019), tannins have demonstrated effectiveness in enhancing tissue repair and reducing inflammation, particularly in promoting diabetic wound healing (Chen et al., 2019). Additionally, studies indicate that tannins can elevate the expression of crucial growth factors, such as vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF-β), essential for tissue repair and regeneration.

Given the significance of their anti-inflammatory activity, particularly in diabetic wounds, which pose a substantial challenge due to impaired healing, these compounds hold promise. In diabetic wounds, inflammation is a key factor contributing to delayed healing. Therefore, the presence of anti-inflammatory compounds is crucial in managing diabetic wounds because they can

Mitigate inflammation, facilitate healing, and prevent the development of chronic wounds.

In recent years, researchers have explored the wound-healing capabilities of *Pentaclethra macrophylla* seeds, yielding promising outcomes (Alonge and Etim, 2015). Tannins, a key component in these seeds, possess astringent and antimicrobial properties, making them beneficial for wound treatment. A study using an aqueous extract of *Pentaclethra macrophylla* seeds on rat wounds revealed substantial improvements in healing time and the quality of healed tissue.

In 14 (Heidari et al., 2019), the wound-healing efficacy of a topical cream featuring *Pentaclethra macrophylla* seed extract was investigated in rats. The findings revealed that the cream facilitated wound contraction and bolstered the tensile strength of the healed tissue, suggesting an expedited and reinforced healing mechanism with pharmacological implications.

Examination of the dichloromethane-methanol extract from *Pentaclethra macrophylla* seed pods revealed the presence of alkaloids, tannins, cardiac glycosides, steroids, and triterpenoids, with tannins and triterpenoids identified as potential contributors to the observed wound healing properties (Kancherla et al., 2019).

Angiogenesis is the formation of new blood vessels, which is an important part of the wound healing process as it helps to deliver oxygen and nutrients to the wound site. Anti-inflammatory compounds promote angiogenesis, which can speed up the healing process 16 (Veith et al., 2019). Cell migration is another essential component of the wound healing process. Cells must move into the wound site to promote healing. Anti-inflammatory compounds can enhance cell migration, which can speed up the healing process. Collagen is the main structural protein in the skin and is essential for wound healing. Anti-inflammatory compounds can stimulate collagen production, which can help to strengthen the skin around the wound and promote healing. Infection is also a significant risk factor for delayed healing and the development of chronic wounds. Anti-inflammatory compounds can help to prevent infection by reducing inflammation, which can create a less hospitable environment for bacteria to grow 17 (Pradhan et al., 2016).

In the context of diabetic wound healing, emollients can play an important role in helping to moisturize the skin around the wound site, which can improve the overall health of the skin and promote healing. In diabetic wounds, the skin around the wound site can become dry and cracked, which can impair the healing process. Emollients can help to moisturize the skin, which can improve its overall health and promote healing. Moisturized skin is also less likely to crack, which can help prevent the development of new wounds. Emollients also help improve skin barrier function, which can help prevent infection and promote healing.

The results obtained from the animal study showed that 1% and 2.5% methanol fractions were comparable with dermazin, whereas 4% of the methanol fraction of the plant extract showed considerable healing properties, which were significantly better compared with the positive control (Dermazin) at $P<0.05$. In addition, the 1% n-Hexane fraction was comparable in wound-healing activity with Dermazin. Again, the 4% n-Hexane fraction of the plant extract showed significant ($p \leq 0.05$) wound healing properties compared with dermazin. This can be attributed to the presence of tannins and triterpenoids present in the plant extract, which has antibacterial and anti-inflammatory activities. The result also showed that 2.5% of the methanol fraction when combined with the drug (glibenclamide) gave a significant ($p \leq 0.05$) wound healing effect against the dermazin-treated group and was comparable with Dermazin and Drug (glibenclamide) treated group.

In addition, gas chromatography-mass spectrometry (GC-MS) to thoroughly assess bioactive compounds in plant-based materials is a widely accepted methodology (Konappa et al., 2020). Analysis of *Pentaclethra macrophylla* seed pods by GC-MS revealed the existence of fifteen (15) chemical compounds, with eight demonstrating pharmacological potential, including E-7-Octadecene with emollient properties, Hexadecanoic acid, methyl ester, and 9,12-Octadecadienoic acid (Z, Z)-, methyl ester with anti-inflammatory activity (Rahmatullah and Hasan, 2016), 10-Octadecenoic acid, methyl ester with both anti-inflammatory and antinociceptive activity, and 12-Methyl-E, E-2,13-octadecadien-1-ol with anticancer, antinociceptive, and emollient properties (Yi et al., 2018).

The study also demonstrated that the methanol fraction was better than the n-hexane fraction following the effects that both gave on different occasions. This result led to the following significant findings.

1. Phytochemical analysis gave phytoconstituents like triterpenoids and tannins, which are responsible for the wound healing properties of the plant.
2. The methanol fraction (polar constituents) showed a more wound healing activity than the n-hexane fraction (non-polar constituents) but was not significant at $p \leq 0.05$.
3. The 4% and 2.5% methanol fraction plus drug was significantly more effective than Dermazin ($p \leq 0.05$).

4. The 4% fraction of the methanol extract demonstrated better wound healing properties compared to Dermazin alone at $p \leq 0.05$. 1% and 2.5% were comparable with Dermazin.
5. The 4% concentrations of n-hexane compared with dermazin also showed better wound healing properties ($p \leq 0.05$). The 1% was comparable with Dermazin.

Conclusion

This research has showcased the wound healing capabilities of the dichloromethane-methanol extract derived from the seed pod of *Pentaclethra macrophylla,* of which 2.5% concentration of the methanol fraction plus drug gave optimum wound healing effect. The extract proved effective in promoting wound contraction and healing compared with the control groups, possibly due to the presence of phytoconstituents like triterpenoids and tannins.

Conflict Of Interest

The authors declare that there are no conflicts of interest.

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Authors Contributions

Johnson-Ajinwo conceptualized/designed the study and wrote the manuscript.
Ikechukwu carried out the research under the supervision of Johnson-Ajinwo.

AUTHOR'S BIOGRAPHY

Okiemute Rosa Johnson-Ajinwo obtained her BSc in Pure and Industrial Chemistry from the Nnamdi Azikiwe University, Awka, Anambra state, Nigeria in 1998 and proceeded to the University of Port Harcourt in 2000, for a Master's degree in Analytical Chemistry. By 2006, she joined the Department of Pharmaceutical/Medicinal Chemistry of the Faculty of Pharmaceutical Sciences in the University of Port Harcourt as a Lecturer 11. The following year, she commenced studies at the University of Nigeria, Nsukka, Enugu state, Nigeria for a Masters' degree in Pharmaceutical Chemistry. By 2017, Rosa obtained her PhD in Pharmacy from the Keele University, UK. In the course of her PhD studies, she attended a number of trainings, conferences and workshops. These included: (i)The Joint pharmaceutical analysis group. Continuing Professional Development for RSC (Royal Society of Chemists) and RPS (Royal Pharmaceutical Society) members. Theme: ICH Q3D (Metals Guide lines): Opportunities and Challenges, 20th October, 2013. (ii) 2013 Bioreactor & Growth Environments for Tissue Engineering Training Course at Keele University, 4th 6th November, 2013. (iii) GxP in Biomedicine' by Univ.-Prof. Dr. Rer. Nat. Habil. Harald G. Schweim (RFWU-Bonn), at the occasion of the PHYTOPHARM 2015 jointly co-organized with Prof. Dr. Jurgen Pomp (Hochschule Bonn Rhein-Seig) and Direktor und Professor Dr. Usfeya Muszznm (former 'Federal Institute for Drugs and Medical Devices' Bonn), 21 -22" July 2015.

Rosa is a beneficiary of five scholarship awards, notably amongst which are: (i) SPDC (Shell Petroleum Development Company), Scholarship award for Undergraduate study, 1995. (ii) McArthur Grant for MSc study, 2008, and (iii) ETF (Education Tax Fund, now known as TETFUND) Scholarship award for PhD study in the UK, 2011. She is also a recipient of several student awards such as; (i) Frank and Mary Loewus Student Travel Award from Phytochemical Society of North America, PSNA, 2015, 54th Annual Meeting. (ii) Best Poster Award from Phytochemical Society of North America, PSNA, 2015, 54th Annual Meeting and (iii) Keele Postgraduate Association, (KPA) Bursary Award, 20th May, 2015.

Currently, she is an Associate Professor and the Acting Head of Department in the Department of Pharmaceutical/Medicinal Chemistry of the Faculty of Pharmaceutical Sciences at the University of Port Harcourt, Rivers State, Nigeria, where she has lectured for close to 17 years. She has published more than 25 papers in reputable journals, supervised over 15 undergraduate projects and 2 post-graduate research works. In

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Her research interest is aimed at obtaining new compounds from medicinal plants which could provide leads for the treatment of inflammatory diseases and cancer. As part of her continuing development in drug discovery, she recently obtained a certificate in Basis of Computer Aided Drug Discovery Part I. Organized by Udemy (Innovative Informatica Technologies), India, 2021. She has co-authored two book chapters: one on ovarian cancer, Titled: Potentials of Phytochemicals and Their Derivatives in the Treatment of Ovarian Cancer, in Handbook on Ovarian cancer: Risk Factors, Therapies and Prognosis. Editor-Bethany R. Colliers and Chemical Characterization, Cytotoxicity Studies and *In vivo* Anti-inflammatory Activities of Anti-cancer Plant: *Rutidea parviflora* (Rubiaceae), in Recent Developments in Medicine and Medical Research Vol. 3, 4 October 2021 Page 38-62. Scholarly Journals she has published in includes: *Molecules, Toxins, Phytomedicine, Journal of Agricultural and Food Chemistry, Bioorganic & Medicinal Chemistry Letters, Annali di Chimica (Now known as ChemSusChem) and Scientia Africana.*

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