Research Article

ISSN 2320-480X
JPHYTO 2022; 11(4): 224-232
July- August
Received: 01-05-2022
Accepted: 18-07-2022
©2022. All rights reserved
doi: 10.31254/phyto.2022.11401

Esther Oluwatoyin Aghaje
Department of Pharmacology, Toxicology and Therapeutics, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Nigeria

Omiyale Olumakinde Charles
Department of Pharmacology, Toxicology and Therapeutics, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Nigeria

Spondias mombin Linn. (Anacardiaceae) Essential Oil Ointment Enhances Healing of Excision Wounds in Rats

Esther Oluwatoyin Aghaje*, Omiyale Olumakinde Charles

ABSTRACT

Background- Wound healing remains a challenging clinical problem, and correct, efficient wound management is essential. Various formulations of Spondias mombin Linn. (Anacardiaceae) is used in the folk medical therapeutics of Africa due to their anti-inflammatory effects and ethnomedicinal claims. Objective- To evaluate the re-epithelialization, rapid wound healing and antioxidant activities of Spondias mombin Linn. (Anacardiaceae) leaves essential oil (SMEO) through excision in vivo model. Materials and Methods- Thirty-eight male rats weighing 250 ± 20g were used. Random grouping into n=6 rats; Group 1 received 50µL of 1% SMEO, Group 2 received 0.1% of DMSO and Tween 20 (Control), Group 3 received Dermazin® ointment, Group 4 was untreated, Group 5 received 50µL of 10% SMEO, Group 6 received 50µL of 15% SMEO, were treated for 14 days. In vivo wound healing rat model was employed with tissues of two rats harvested per group on the 3rd, 10th and 14th days after excision for histological analysis. The SMEO of (25–100 µg/ml) was passed through DPPH, Nitric oxide, Reducing power assays. Results- The antioxidant assays showed scavenging of species in close comparison with standard in a dose dependent manner. The essential oil showed promising results even at low concentration of 1%. The 10% and 15% wound contraction progression showed efficiency over the standard. Macroscopic observation and Histological analysis revealed a significant wound healing process of the treatment groups compared to the vehicle-treated and unwounded controls, after the 3rd, 7th and 14th day. Conclusion- The essential oil showed ability to initiate re-epithelization, proliferative stimulation of new blood vessels, collagen fiber synthesis and overall improved wound healing better than the standard (Dermazin®), therefore, a possible presentation as lead for drug development.

Keywords: Wound healing, Spondias mombin, Anti-inflammatory, Cytokines, Essential oil, Antioxidant assay.

INTRODUCTION

The massive reports of different adverse effects of chemical drugs today are a growing problem in therapeutics. This has made the exploration of suitable natural drug candidates as alternatives with little or no adverse effects [1-2]. Reports of Spondias mombin Linn. (Anacardiaceae), referred to as Hog plum, Iyeye (Yoruba), and Ijikara (Igbo). Its fruit is said to contain vitamin C with its leaves usually odd-numbered [3-5]. Its applications in ethnomedicine as remedy for throat infections and holes in the teeth; aid as post-partum relieve for women. In South America, wound aid patch is made from either fresh or dried leaves for faster healing. Concotions of the leaves and flowers are said to relieve stomach-ache, ameliorate gastric ulcers, urethritis, cystitis and cure diseases transmitted through sex such as gonorrhea [6-9]. A recent paper by Ishola et al., 2017 reported some of its protective roles against dementia [10].

The biological activities of the essential oil of Spondias mombin holds unresearched potentials even as the leaves, stem, flowers and fruits activities and usefulness have been thoroughly researched. The complicated process of wound healing, involving multi-steps, stages and mediators is designed to re-initiate hemoeostasis [11,12]. The use of penicillin topical gel, ampicillin to counter microorganism invasion during this delicate process holds its disadvantages such resistance which calls for a strong concern for immediate resolution [11,2].

Some natural plant candidates are however being discovered, re-discovered or re-purposed everyday due to their therapeutic composition such as phenolics, proven to interfere favorably in cellular activities of the inflammatory and proliferative steps of wound healing and can be said to hold great potentials [13-17].

Spondias mombin Linn. (Anacardiaceae) essential oil contains monoterpene (about 22.5%) and sesquiterpene (about 48.5%). These include Beta-caryophyllene (19.1-30.5%), caryophyllene oxide (5.5%) and u-humulene (3.5%) and others [18-20]. The activities of this natural sequiterpene, beta-caryophyllene as wound healing lead works through reduction of inflow of inflammatory cytokines by blocking of the Toll receptor and CD14 receptor [21,22]. Its does this through its receptors: CB1 and 2 (expressed mostly in the epidermis region of the skin) [23,24].
This study was designed to evaluate Spondias mombin leaf essential oil for its wound healing activities (in vivo) in rats and to identify the chemical compounds responsible for activities of Spondias mombin as natural drug lead.

**MATERIALS AND METHODS**

**Plant collection and Essential oil Extraction**

*Spondias mombin* Linn. (Anacardiaceae) leaves were sourced from the herbal market in Mushin, Lagos state, Nigeria. This was identified and authenticated as *Spondias mombin* Linn. (Anacardiaceae) by Mr Adeleke Tijani Isaac, Department of Pharmacognosy, College of Medicine, University of Lagos, Nigeria. These *Spondias mombin* leaves were prepared by thorough washing with distilled water. These were air-dried and then pulverized into fine powder. About 400 g out of 1200 g at a time were hydrodistilled in Clavenger glass equipment (Borosil, India) for 4 hours. 0.35% (v/w) essential oil yield was recorded.

**Ointment preparation**

*Spondias mombin* essential oil dilution: 1%, 10% and 15% solution dissolved in 0.1% DMSO (Sigma-Aldrich Inc., St. Louis, MO) and topped up with Tween 20 (Sigma-Aldrich Inc., St. Louis, MO).

**Chemicals and Biochemicals**

DMSO (Sigma-Aldrich Inc., St. Louis MO), Tween 20 (Sigma-Aldrich Inc., St. Louis, MO), DPPH, FeCl₃, sulphanalimide, naphthylenediamine dichloride and orthophosphoric acid, ascorbic acid, sodium nitroprusside, Folín–Ciocalteu reagent, NaNO₂, AlCl₃, TCA, Phosphate buffer. Other reagents are of analytical grade.

**Laboratory Animals**

Adult male albino rats (n=38, 250 ± 20 g) were acclimatized for a week, subjected to standard and ethical laboratory conditions at 23± 2°C without tampering with the normal 12 hours day time cycle and freely fed standard diet (Animal share feeds, grower, Ogere-Remo. Ogun state, Nigeria) and access to water. Ethical approval was obtained from Animal Care and Use Research Ethics Committee (ACUREC) with College of Medicine HREC number: CMUL/HREC/06/21/899. All protocols and guidelines concerning animal care and handling was duly observed in all phases of the work and conforms with the Helsinki Declaration 1975 (as amended) and The Institutional Animal Care and Use Committee (IACUC).

**Animal grouping**

Grouping was based on weight comparison:

1- rats treated with 1% of essential oils from leaves (50µL per animal). 2- rats treated with a solution containing 1% DMSO and Tween 20 (Control). 3- rats treated with standard drug (Dermazin® ointment). 4- rats left untreated 5-rats treated with 10% of essential oils from leaves (50µL per animal). 6- rats treated with 15% of essential oils from leaves (50µL per animal) with the remaining two animals as unwounded samples.

**Wound healing activity**

*In Vivo Wound Healing Experiments:*

The dorsal air of the rats was shaved. An excision of a 2 cm was punched at shaved back. Application of 50 µl of each specified treatment ointment was done after wound creation and also every day for 14 days. The application of the essential oil was done without using essential oils or bases for the purpose of this experiment. Two rats were sacrificed on the 3rd, 10th and 14th day after wound creation and newly generated skin tissue was excised for further histomorphological examination, and cytokines/gene expression analysis.

**Wound Healing Rate Determination:**

Wound contraction was recorded immediately after excision, the on the 3rd, 7th, 10th, 12th and 20th days with a camera and a caliper.

Wound healing rate = (wound area on day 0 − wound area on day n) / (wound area on day 0) × 100%, where n = 0, 3, 7, 10, 12, 14 days post-wounding. Values was expressed as the percentage of wound area reduction.

**Tests and Assays**

**Histomorphological Analysis:**

Tissues collected were immersed for 4 hours in paraformaldehyde (4%), passed through Isopropanol (100%) and subsequently passed through normal histology standard procedures. Microns of 3 µm sized tissues was stained conventionally with Hematoxylin and Eosin to observe neovascularization, inflammation progression and other histological parameters. This was done with a microscope (Oleica microsystems, Germany). Assessment criteria used were epidermal regeneration, granulation tissue score, blood vessels regeneration, inflammation score and collagen fibre arrangement. This is based on an improved method devised from the works of.

**Antioxidant Assays**

**Diphenyl-1-picrylhydrazyl assay:**

[31] was followed to quantify the capacity of the essential oil to inhibit antioxidants. Specific dilutions of the sample between 25 and 100 µg/ml were mixed with 5ml of Diphenyl-1-picrylhydrazyl dissolved in 0.004% methanol. Incubation of this mixture was done for 30 minutes. The spectrophotometer value was recorded in replicates with a standard comparison of Ascorbic acid. IC % = [(A0 − At/A0) × 100, where A0 and At are the absorbance values of the control and test sample, respectively. This was plotted against concentration, and the equation for the line was used to obtain the IC50 value.

**Nitric Oxide Assay:**

[32] was followed. Exactly two microliters of 10mM Na₂[Fe(CN)₅NO]₂H₂O in Cl₂H₃K₂Na₃P₂ of 7.4 potential of hydrogen value was pipetted and mixed with dilutions of the isolated essential oil between 25 and 100 µg/ml. This was incubated for 2 hours, 30 mins at room temperature. Followed by addition of Griess reagent of one microliter and two microliter H₃PO₄. The solution was read with a spectrophotometer at the proper wavelength. "Inhibition of free radicals by Nitric Oxide was calculated by:

Percent Nitric oxide scavenging capacity (IC %) = (A0 − At/A0) x 100

Where, A0 and At are the absorbance values of the control sample and the test sample, respectively. The inhibition % was plotted against concentration, and the equation for the line was used to obtain the IC50 value.

**Reducing power:**

This was determined with a process by [33]. To start with, 2.5mL of 1% potassium hexacyanoferrate [K₃Fe(CN)₆], 2.5 µL of 0.2 M PBS (pH 6.6) and specific concentrations (25−100 µg/mL) of essential oil extract suspended in 1mL of distilled water and incubated at 50°C for 20 min. Thereafter, 2.5 µL of trichloroacetic acid was added to the mixture. This was centrifuged at 400 rpm for 10 min after which 2.5 µL of the supernatant was mixed with an equal amount of distilled...
water and 0.5mL of 0.1% FeCl₃. End solution’s absorbance determination was carried out to a blank at 700 nm.

**Statistical Analysis**

Statistical analysis was carried out using GraphPad Prism 5.01 Software. All experimental measurements were carried out in triplicate, expressed as average of three analyses (Mean±SEM) and analyzed as *p<0.05 and **p<0.01 and ***p<0.001 vs vehicle/standard, using one way/two-way ANOVA followed by Bonferroni posttests or Tukey Multiple comparison test.

**RESULTS**

**Antioxidant Assay Results**

The oil was analysed with ascorbic acid as standard to determine its relative potency. The *Spondias mombin*’s volatile oils scavenged DPPH in close efficiency behind the standard used (ascorbic acid) in all doses. The highest concentration of 100µg/ml of the standard scavenged 83.25 µg/ml with *Spondias mombin* following closely at 73.19 µg/ml. The oil fraction examined was highly active, with IC₅₀ value of 58.2 compared to the standard with a slightly better value of 29.32 (Figure 1, 2, 3).

The capability of the two oils to reduce K₃Fe(CN)₆ significantly shows its effectiveness to halt the oxidation of cellular macromolecules by oxidizing molecules in wounds etc. Also, the reducing effect of the oils was similar to that produced by vitamin C.

In the Nitric oxide assay, the essential oil exhibited 65.3 IC₅₀ value in comparison with the standard at 33.9 IC₅₀ value (Figure 4, 5, 6).

**Diphenyl-1-picrylhydrazyl assay:**

**Figure 1:** Graph of Diphenyl-1-picrylhydrazyl assay for *Spondias mombin* essential oil and ascorbic acid standard

**Figure 2:** Scatter plot of *Spondias mombin* essential oil Diphenyl-1-picrylhydrazyl assay IC₅₀ (value of 58.2).

**Figure 3:** Scatter plot of Ascorbic acid standard DPPH assay IC₅₀ (value of 29.32)

**Nitric Oxide Assay**

**Figure 4:** Graph of *Spondias mombin* essential oil Nitric acid assay

**Figure 5:** Scatter plot of *Spondias mombin* essential oil Nitric acid assay IC₅₀ (value of 65.3)

**Figure 6:** Scatter plot of Ascorbic acid Nitric acid assay IC₅₀ (value of 33.9)
The Journal of Phytopharmacology

Reducing Power Assay

Figure 7: Graph of Spondias mombin essential oil Reducing power assay

The Wound Contraction For 14 Days

The Spondias mombin Linn. essential oil showed ability to initiate re-epithelialization, tissue remodeling and improved wound healing even at low concentration of 1% as shown in the table I, where the essential oil group showed moderate improvements above the blank and untreated groups on day 7 through to 12. Even though the 1% essential oil group had a slow significant initial effect as compared to the standard from day 3 through 12, it made a full recovery on the 14th day with the essential oil group’s wound diameter reaching the 0 cm mark before the standard dermazin® ointment. (0.2 cm).

On keen observation, the 1%, 10% and 15% essential oil gave a complete wound closure with the puncture hole undistinguishable to the human eye from inside out when the healed skin was excised, confirming a better scar appearance.

The 10 % and 15 % progression showed a close association with the standard from the beginning with very significant healing potential over the blank and untreated before overtaking the standard on the 12th day and a run to 100 % contraction with the smallest scar.

Table 1: Table showing the mean and percentage of wound contraction for each treatment group after 14 days

<table>
<thead>
<tr>
<th></th>
<th>Blank (cm)</th>
<th>Untreated (cm)</th>
<th>Essential oil (1%) (cm)</th>
<th>Essential oil (10%) (cm)</th>
<th>Essential oil (15%) (cm)</th>
<th>Standard drug (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>2.00 (0%)</td>
<td>2.00 (0%)</td>
<td>2.00 (0%)</td>
<td>1.67 (0%)</td>
<td>1.50 (0%)</td>
<td>2.00 (0%)</td>
</tr>
<tr>
<td>Day 3</td>
<td>1.90 (5%)</td>
<td>2.00 (0%)</td>
<td>1.96 (2%)</td>
<td>1.65 (1.2%)</td>
<td>1.30 (13%)</td>
<td>1.76 (12%)</td>
</tr>
<tr>
<td>Day 7</td>
<td>1.73 (14%)</td>
<td>1.90 (5%)</td>
<td>1.63 (19%)</td>
<td>1.18 (30%)</td>
<td>0.98 (35%)</td>
<td>1.10 (45%)</td>
</tr>
<tr>
<td>Day 10</td>
<td>1.60 (20%)</td>
<td>1.23 (39%)</td>
<td>1.03 (49%)</td>
<td>0.53 (69%) **</td>
<td>0.50 (67%) *</td>
<td>0.60 (70%)</td>
</tr>
<tr>
<td>Day 12</td>
<td>1.35 (33%)</td>
<td>1.00 (50%)</td>
<td>0.45 (78%) *</td>
<td>0.10 (94%) ***</td>
<td>0.25 (83%) **</td>
<td>0.30 (85%)</td>
</tr>
<tr>
<td>Day 14</td>
<td>0.65 (70%)</td>
<td>0.65 (68%)</td>
<td>0.00 (100%) ***</td>
<td>0.00 (100%) ***</td>
<td>0.00 (100%) ***</td>
<td>0.20 (90%)</td>
</tr>
</tbody>
</table>

The mean and percentage of wound contraction for each treatment group treated with vehicle, 1% Spondias mombin essential oil, 10% Spondias mombin essential oil and the standard drug, Dermazin® after 14 days. *p<0.05 and **p<0.01 and ***p<0.001 vs vehicle/standard, using two-way ANOVA followed by Bonferroni post-tests.

Histological Analysis Results

The tissue slides are interpreted in tabulated summary according to the groups

Evaluation of wound healing was based on the assessment of epithelialization, epidermal differentiation, amount of granulation tissue, inflammation, collagen fiber orientation, and neovascularization. Total histological score was finally obtained by adding scores of different assessed parameters as shown in Table 2 below. The higher concentrations of Spondias mombin L. essential oil showed efficiency in histological scores against the vehicle and standard, with the 10% essential oil showing statistical significance over the vehicle;

Table 2: Key to Modified histological scoring system from Clinical parameters of cutaneous lesions. (This gives clarity to Table 3 below)

<table>
<thead>
<tr>
<th>Score</th>
<th>Epithelialization</th>
<th>Differentiation</th>
<th>Amount of granulation tissue</th>
<th>Inflammation</th>
<th>Collagen fiber orientation</th>
<th>Neovascularization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 1</td>
<td>Absent</td>
<td>Absent</td>
<td>Profound</td>
<td>Severe</td>
<td>Vertical</td>
<td>&lt;5hpf</td>
</tr>
<tr>
<td>Score 2</td>
<td>Moderate</td>
<td>Present</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Mixed</td>
<td>6-10hpf</td>
</tr>
<tr>
<td>Score 3</td>
<td>Marked</td>
<td>Absent</td>
<td>Weak</td>
<td></td>
<td>Horizontal</td>
<td>&gt;10hpf</td>
</tr>
</tbody>
</table>

hpf- high power field

Score- Individual and Total epithelialization, epidermal differentiation, amount of granulation tissue, inflammation, collagen fiber orientation, and neovascularization scores.

Amount of granulation tissue- If observed parameters indicate Profound, Moderate or Absent granulation.

Inflammation- If observed parameters indicate Severe, Moderate or Weak Inflammation.
Wound Contraction Results (After 14 Days)

i. Day 0

![Wound Contraction Results (Day 0)](image)

ii. Day 3

![Wound Contraction Results (Day 3)](image)

iii. Day 7

![Wound Contraction Results (Day 7)](image)

iv. Day 10

![Wound Contraction Results (Day 10)](image)

v. Day 12

![Wound Contraction Results (Day 12)](image)

vi. Day 14

![Wound Contraction Results (Day 14)](image)

Figure 8 (i-vi): Pictures showing the Wound Contraction Results (After 14 Days) of (A) Blank (B) Untreated (C) Essential oil 1% (D) Essential oil 10% (E) Essential oil 15% (F) Standard (Dermazin®)
**Table 3:** Modified histological scoring system for each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>Epithelialization Score</th>
<th>Differentiation Score</th>
<th>Amount of granulation tissue Score</th>
<th>Inflammation Score</th>
<th>Collagen fiber orientation Score</th>
<th>Neovascularization Score</th>
<th>Total Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Essential Oil</td>
<td>Day 3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Day 10</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Vehicle</td>
<td>Day 3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Day 10</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Standard (Dermazin®)</td>
<td>Day 3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Day 10</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Untreated</td>
<td>Day 3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Day 10</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>10% Essential Oil</td>
<td>Day 3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>15*</td>
</tr>
<tr>
<td></td>
<td>Day 10</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>15% Essential Oil</td>
<td>Day 3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Day 10</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>16</td>
</tr>
</tbody>
</table>

*p<0.05 vs vehicle, using ANOVA followed by Tukey Multiple Comparison post-tests

**Figure 9:** Graphical illustration of Histological Scoring for Inflammation on different days. (Reversed to depict value 1 as lowest and 3, as highest to illustrate reduced inflammatory cells as shown)
Figure 10: Histomorphological Analysis: Plate I- Medium power photomicrograph of normal rat skin- control (h & e x100). Plate II- Low power photomicrograph of the skin treated with 1% essential oil, day 3 showing profound granulation tissue and severe inflammation (white arrow head). (h & e x40). Plate III- Low power photomicrograph of the skin treated with 1% essential oil, day10 showing profound granulation tissue and severe inflammation (white arrow head). (h & e x40). Plate IV- Low power photomicrograph of the skin treated with 1% essential oil, day14. (h & e x40). Plate V- Low power photomicrograph of
the skin treated with Control (Vehicle) day3. (h & e x40). Plate VI- Low power photomicrograph of the skin treated with Control (Vehicle), day14. (h & e x40). Plate VIII- Low power photomicrograph of the skin treated with Standard (dermazin), day10. (h & e x40). Plate 3- Low power photomicrograph of the skin treated with Standard (dermazin), day5. (h & e x40). Plate IX- Low power photomicrograph of the skin treated with Standard (dermazin), day14. (h & e x40). Plate XI- Low power photomicrograph of the wounded untreated skin, day3 showing profound granulation tissue and severe inflammation (white arrow head), (h & e x40). Plate XII- Low power photomicrograph of the wounded untreated skin, day14. (h & e x40). Plate XIV- Low power photomicrograph of the wounded untreated skin, day14. (h & e x40). Plate XV- Low power photomicrograph of the wounded skin treated with 10% essential oil, day10. (h & e x40). Plate XVI- Low power photomicrograph of the wounded skin treated with 10% essential oil, day14. (h & e x40). Plate XVII- Low power photomicrograph of the wounded skin treated with 15% essential oil, day14. (h & e x40). Plate XIX- Low power photomicrograph of the wounded skin treated with 15% essential oil, day14. (h & e x40). H & E – Hematoxylin and Eosin

**DISCUSSION**

Herbal plants serve as alternatives to chemical counterparts [34]. Several advantages have been observed such as improved healing and absence of adverse effects and situations like drug resistance [36,35]. *Spondias mombin*’s leaves, stem, bark efficacy has been proven overtime [3]. This report serves to state the beneficial effects of its essential oil on wound healing through its Cytokines modulatory activities.

Rats treated with *Spondias mombin* essential oil ointment have shown better wound contraction than those of the control group, even though the 1% essential oil group had a slow significant initial effect as compared to the standard from day 3 through 12, it made a full recovery on the 14th day with the essential oil group’s wound diameter reaching 100% wound contraction before the standard Dermazin® ointment (90% wound contraction).

The 10% essential oil group outperformed the Dermazin® ointment group with 94% trailed by the 15% at 83% contraction on the 12th day against the standard’s contraction of 85% progressing with 100% contraction on the 14th day against the standard’s 90% wound contraction. This increase in contraction rate can be explained by a shortening of the inflammation-dominated wound healing stage due to increased cells stimulation, antimicrobial activity of the plant against interfering pathogen invasion and subsequent rapid re-epithelization and wound closure [7,9]. These effects on the process have resulted in a shortening of the epithelialization period and after 14 days the wounds were completely covered with new skin and went further to display a better scar appearance than the standard Dermazin® cream experimental group. The results of this study are similar to the reports that evaluated the effect of the leaves and barks of *Spondias mombin* and also effects of Lavender plant essential oil on wound healing as no publication was found to have researched the wound healing properties of the essential oils of *Spondias mombin* leaves [3,6].

Examination of Hematoxylin and Eosin-stained rat wound tissues from the various days confirms the *in vivo* results as it has revealed that regeneration/re-epithelization was much more rapid in the treated group compared to control group. The histological examination revealed improved epithelial scores and regeneration of the epidermis, weak inflammation score, increased neovascularization of greater than 10 neovascularization per high power field (hpf) due to *Spondias mombin* leaves’ essential oil essential application. Reduced inflammation score observed in *Spondias mombin* leaves’ essential oil treated rats wound tissues in control comparison, most probably linked to the anti-inflammatory plant action, leading to the reduction of inflammatory cells around the wound site.

Histopathology results are in agreement with reports from [14,37] that reported their histopathological findings on Dragon blood ointment as a standard and *Lawsonia inermis* respectively. The therapeutic effects can be linked to the components of the essential oil. Such as sequiterpenes (Beta carophyllene), alkaloids, phenolics and flavanoid content [20-22, 38,39]. Beta-Caryophyllenes can also be found in essential oil of some wound healing plants such as *Piper nigrum* called Black Pepper and *Melissa officinalis*.

Alkaloids (inhibits arachidonic acid synthesis), phenolics and flavanoids have been proven to enhance antioxidation, help reduce microorganisms on wound site and help create conditions for regeneration of fibroblasts [40]. Which can be linked to wound antioxidation and increase in Superoxide Dismutase enzymes to provide healing effects [41]. The results of the antioxidante/quantitative phytochemical estimation (DPPH and Total antioxidant assay etc.) point to the positive effects of the free radicals mopping activities of *Spondias mombin* essential oil on reaction where the electron is donated to them. They were noted to possess a high level ofthis ability which may be attributed to the strong hydrogen donating ability of the phenols. An important property that combats the start or progression of oxidative stress [10] which can be attributed to *Spondias mombin* essential oil’s high antioxidant activities especially when it showed close properties to the standard used, Ascorbic acid. which can be attributed to *Spondias mombin* essential oil’s high antioxidant activities especially when it showed close properties to the standard used, Ascorbic acid. Further studies on gene expression and cytokines analysis are recommended to be carried out to decipher the complete mechanism of action of *Spondias mombin* essential oil on wound healing.

**CONCLUSION**

The essential oil showed ability to initiate re-epithelization, proliferative stimulation of new blood vessels, collagen fiber synthesis and overall improved wound healing better than the standard (Dermazin®), achieving regeneration faster and giving a functional and aesthetic scar. Therefore, a possible presentation as lead for drug development.

**Acknowledgements**

My profound gratitude goes to my supervisor, Prof (Mrs) Oluwatoyin Agbaje for her guidance. We have this piece due to her efforts.

**Author contribution**

Omiyale Olumakinde Charles- Calendar months effort, conducted the antioxidant experiments and assays, wound healing experiments, gene expression, data analysis and manuscript writing. Prof. Oluwatoyin Esther Agbaje- Calendar months efforts. Supervised the entire project, including experimental design, all experiments, analysis and interpretation of data gathered, manuscript writing supervision.

**Conflict of Interest**

None declared.

**Financial Support**

None declared.

**REFERENCES**

2. Barku VY. Antioxidant and wound healing properties of some selected plants from kpando traditional area: Isolation of flavonoids from

![](image)
Anogeissus leiocarpus (dc) Guill and Perr (combretaceae) (Doctoral dissertation, University of Cape Coast).


HOW TO CITE THIS ARTICLE


Creative Commons (CC) License-

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license. This license permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. (http://creativecommons.org/licenses/by/4.0/)

The Journal of Phytopharmacology