The Journal of Phytopharmacology 2022; 11(4):260-267
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2022; 11(4): 260-267
July- August
Received: 01-05-2022
Accepted: 08-06-2022

doi: 10.31254/phyto.2022.11406

Esther Oluwatoyin Agbaje
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

Correspondence:
Dr. Esther Oluwatoyin Agbaje
Department of Pharmacology, Toxicology and Therapeutics. Faculty of Basic Medical Sciences. College of medicine. University of Lagos. Nigeria
Email: estheragbaje01@gmail.com

Anti-inflammatory and Cytokines Modulatory Activities of *Spondias mombin* Linn. (Anacardiaceous) in Wound Healing: Roles of IL6

Esther Oluwatoyin Agbaje, Omiyale Olumakinde Charles

ABSTRACT

Health problems relating to wound healing remain a significant issue in health management. Various formulations of *Spondias mombin* Linn. (Anacardiaceous) serves potent roles in ethnomedicine.

To explore the counter-inflammatory and cytokines modulatory roles together with the antioxidant and wound healing properties of *Spondias mombin* Linn. (Anacardiaceous) leaves essential oil (SMEO) on excised wounds.

Spondias mombin Linn. (Anacardiaceous) leaves essential oil was isolated through hydro distillation essential in Clevenger type apparatus (Borosil, India). Then thirty-eight healthy adult male albino rats (250 ± 20g) were grouped randomly into (n=6); Group 1- 50 µL 1% SMEO, Group 2- 0.1% DMSO and Tween 20 (Control), Group 3- Dermazin® ointment, Group 4-untreated, Group 5-50µL 10% SMEO, Group 6- 50µL 15% SMEO, with two unwounded samples, were treated for 14 days. Tissues of two rats was harvested per group on the 3rd, 10th and 14th days after excision, wounded areas were excised for RT-qPCR cytokines analysis and gene expression (Light Cycler, Mannheim, Germany) (IL 6, IL1β, TNFα). The SMEO of (25–100 µg/ml) was passed through Total antioxidant/flavonoid/phenol phytochemical estimation assays.

The assays showed high presence of flavonoids/phenols. The 10% and 15% essential oil healing progression showed efficiency over the standard. Cytokine analysis/gene expression revealed a significant reduction in IL6 levels, a key regulator of other pro-inflammatory cytokines and reparative process, after the 3rd, 7th and 14th day.

The extracted *Spondias mombin* Linn. essential oil showed stimulation of an anti-inflammatory effect through a significant reduction of IL-6 levels as compared to the vehicle and standard treatment on the 3rd, 10th through to the 14th day of the treatment course, neo-vascularization, tissue regeneration phase and overall improved wound healing better than the standard.

Keywords: Wound Healing, *Spondias mombin*, Anti-Inflammatory, Cytokines, Essential Oil, Antioxidant Assay.

INTRODUCTION

The search for natural drug leads is unending in science as a result of different adverse effects reports of conventional synthetic drugs [1,2]. *Spondias mombin* Linn. (Anacardiaceous) has numerous local applications. It is referred to as Hog plum, Iyeye (Yoruba), Ijikara (Igbo), Its fruit is said to contain vitamin C with its leaves usually odd-numbered [3-5].

The leaves extract is commonly used as passed down, local remedies such as, throat diseases and cavities; aid in women who just gave birth. (Nwogu *et al*, 2007) The juice from the leaves and dried, crushed ones are also used to aid wound healing by direct application on the affected area. This is popular in South America. Herb portions containing its parts such as flowers are believed to cure stomach-ache, gastric ulcers and Sexually Transmitted Diseases [6-9]. Numerous studies have been carried out on the leaf, fruit, flower, and stem bark of *Spondias mombin*, not much has been revealed about the biological activities of its essential oil. Therefore, this recent study has been geared towards the anti-inflammatory and wound healing activities of *Spondias mombin* leaves essential oil.

Wound healing is stepwise, involving various stages and numerous mediators with a sole aim of return to homeostasis, from blood coagulation, inflammation, through to neovascularization and epithelialization. The success of this long and orderly process depends on many biochemical processes, cells and

mediators involved [9,10]. One of these is IL 6, a proinflammatory cytokine with many functions in inflammation, modulation of other proinflammatory cytokines (TNFα and IL1β), response to immune signaling and blood cells formation. Some studies have shown that drug candidates that can modulate IL 6, help promote better wound healing. Tackling wound invasion by disease-causing organisms to aid wound healing requires application of drugs such as penicillin topical gel, ampicillin. Other drugs used in wound healing may include collagen-degrading enzymes for treatment by debriding of necrotic tissues of infected wounds and thereby achieving re-epithelization fast, growth factors such as VEGF, TGFβ, Corticosteroids and other recent products [11]. Even though they are effective, it is of urgent concern that the cases of antimicrobial resistance and issues with other chemical components are on the increase and alternatives must be found [12,13].

Various plants have however been showing promising results as drug leads [13-15]. This is connected to the presence of phenolic contents, tannins, terpenes and other secondary metabolites in their composition that modulate wound healing at appropriate stages. Plant candidates have been proven to shorten wound healing phases and initiate re-epithelialization faster and therefore shows greater promises [16,17]. *Spondias mombin* essential oil contains monoterpene (22.5%) and sesquiterpene (48.5%). Such as Beta-caryophyllene (19.1-30.5%), caryophyllene oxide (5.5%) and α-humulene (3.5%) just to mention a few [18-20]. Some studies have pointed to the activities of β-caryophyllene (a natural sesquiterpene) as wound healing candidate through reduction of inflow of inflammatory cytokines by blocking of the Toll receptor and CD14 receptor [21,22]. Its receptors have been uncovered as CB1 and 2 (expressed in the epidermis region of the skin) [23,24].

This study primarily seeks to explore *Spondias mombin* leaf essential oil for its natural, potent, anti-inflammatory and wound healing activities on wounds using excision wound model in rats and to identify the intrinsic chemical compounds responsible for the anti-inflammatory, antioxidant, wound healing activities and possible mechanism of action of *Spondias mombin* as natural drug candidates.

MATERIALS AND METHODS

Ethical Approval and Plant Identification

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 duel 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). *Spondias mombin* L. leaves were purchased from a local market in Mushin. Lagos state. The plant was identified as *Spondias mombin* Linn. (Anacardiaceous) by Mr. Adeleke Tijani Isaac of the Department of Pharmacognosy Medicinal Garden, College of Medicine. University of Lagos.

Chemicals and Biochemicals

The following chemicals and kits were used: DMSO (Sigma-Aldrich Inc., St. Louis, MO), Tween 20 (Sigma-Aldrich Inc., St. Louis, MO), TCA, Phosphate buffer, RNeasy micro kit, Qiagen, FIRE Script RT cDNA Synthesis MIX with Oligo (dT) and Random primers for TNF-α, IL-6 and IL-1β cytokines and β-actin control (Solis Bio Dyne. Estonia). Other reagents are of analytical grade.

Extraction of the essential oils from leaves

Collected *Spondias mombin* leaves were washed and was further run through distilled water. The leaves were air-dried and then ground. These leaves were (400 g out of 1200 g per session) were passed through hydro distillation in Clevenger equipment (Borosil, India) for 4 hours following [25]. The extraction yielded oil of 0.35% (v/w).

Ointment preparation

Spondias mombin essential oil was made into a (1 %, 10 % and 15 %) solution with 0.1 % DMSO (Sigma-Aldrich Inc., St. Louis, MO) and Tween 20 (Sigma-Aldrich Inc., St. Louis, MO).

Animals

Thirty-eight healthy adult male albino rats (experiment worthy) of weights ranging from 250 ± 20 g were kept under adaptive feeding for about a week and also subjected to standard and ethical laboratory conditions at 23± 2°C without tampering with the normal 12 hours day time cycle and fed freely standard diet with standard feed (Animal share feeds, grower, Ogre-Remo. Ogun state. Nigeria) and water.

Grouping of animals

Animals were distributed into groups based on their weights. As follows:

GROUP 1- rats treated with 1% of essential oils from leaves (50 µL per animal)

GROUP 2- rats treated with a solution containing 0.1% DMSO and Tween 20 (Control)

GROUP 3- rats treated with standard drug (Dermazin® ointment)

GROUP 4- rats left untreated

GROUP 5-rats treated with 10% of essential oils from leaves (50 µL per animal)

GROUP 6- rats treated with 15% of essential oils from leaves (50 µL per animal) with the remaining two animals as unwounded samples.

Wound healing activity

In Vivo Wound Healing Experiments

The hair on the back of the rats was shaved. Then a 2cm excision wound was created on the shaved spot. About 50 µl of each solution was given after excision and then every day for 2 weeks [26]. The diluted essential oil was applied (including control) without any addition of other oils or cream bases so as to factor out any interference [27]. Euthanasia of 2 rats on the 3rd, 10th and 14th day post excision and healing tissue was collected for further histological examination. and cytokines analysis.

Wound Healing Contraction Determination

Reducing wound contraction on the excision day, 3rd, 7th, 10th, 12th and 12th days was recorded with a camera, a caliper was used for the closure rate.

Wound healing contraction = (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 [26].

Tests and Assays

Quantitative Real Time Polymerase Chain Reaction Cytokine Gene Expression Measurements

Healing wound tissues were routinely stored in 0.02 Molar Hosphate Buffer (pH 7) and stored under 80°. TNF-α, IL-6**,** IL1-β were assayed with qPCR.

RNA Isolation/Purification

Exactly 0.25 g excision from the wounds was weighed with a

weighing balance into a microcentrifuge tube and ground in RNA later (Qiagen, ON, Canada). 500 µL of the Lysis Buffer (RNeasy micro kit, Qiagen), was added, vortexed for 5 minutes, incubated, Then, 300 µL of Isopropanol was pipetted into it and vortexed again (Eppendorf, Germany). The mixture was then centrifuged in a spin column (Eppendorf, Germany) at 10,000 rpm for some seconds. The part that flowed through this spin column was removed. 700 µL of secondary wash buffer was added to the spin column and centrifuged at 10,000 rpm for 30 seconds. The flow-through was again discarded and the collection tube blotted on tissue. Ccentrifuge action at 12,000 rpm, 2 minutes was done to remove wash buffer, a this is transferred to a new tube then a pipette of 50 µL of Elution Buffer (RNeasy micro kit, Qiagen), was done and incubateion carried out at room temperature, for a minute, this was centrifuged at 10,000 rpm for 1 minute. The trapped RNA is released and stored under 80° [28].

Complementary DNA synthesis

A 40x cDNA mix was prepared containing 40 µL template RNA, 40 µL 10x RT reaction premix with with Oligo (dT) and random primers, 30 µL RT enzyme and Risograph RN as inhibitor and made up with 250 µL nuclease free water (Solis Bio Dyne. Estonia). 9 µL of the mix was pipetted to 1 µL of the template RNA. The plate was slightly (at 30% of maximum speed) vortexed (Eppendorf, Germany) and centrifuged (Eppendorf, Germany) for 30 seconds at 1,000 rpm. Prsetting of the thermocycler (Light Cycler, Mannheim, Germany) was fixed at 45 cycles, 95℃ for 20 seconds, matter annealing temperature for 4–5 seconds and 72°C for 8 seconds. The melt-curve was documented for amplification monitoring. (Solis Bio Dyne. Estonia) [28].

Quantitative Real Time Polymerase Chain Reaction Protocol

A 45x qPCR mix was prepared containing 90 µL Master mix, 45 µL cDNA, 9 µL primer of both the 3' and 5' and *β*-actin control, 9 µL of probe (SYBR green) and 306 µL of nuclease free water (Solis Bio Dyne. Estonia), vortexed (Eppendorf, Germany) and centrifuged for 30 s at 1,000 rpm. Then the thermocycler (Light Cycler, Mannheim, Germany) was fixed at 45 cycles, 95℃ for 20 seconds, matter annealing temperature for 4–5 seconds and 72°C for 8 seconds.

Melt curve was programmed from 55-80°C. The Ct value was gotten and the ΔCt, ΔΔCt and RQ were derived from the following equations:

Delta Ct = Ct gene test – Ct endogenous control

Delta Ct = ΔCt sample1 – ΔCt calibrator

Relative quantification (RQ) = 2-ΔΔCt [28].

The determinant calibrator helps determine the RQ of all samples as they are placed side by side with it. A relative quantification of 10 shows that the particular gene is 10 times present.

Quantitative Real Time Polymerase Chain Reaction Primers Used

IL 1β forward

5-CAC CTC TCA AGC AGA GCA CAG-3

IL 1β reverse

5-GGG TTC CAT GGT GAA GTC AAC-3

IL 6 forward

5-TCC TAC CCC AAC TTC CAA TGC TC-3

IL 6 reverse

5-TTG GAT GGT CTT GGT CTT TAG CC-3

TNFα forward

5-AAA TGG GCT CCC TCT CAT CAG TTC-3

TNFα reverse

5-TCT GCT TGG TTT GCT ACG AC-3

β-actin forward

5-AAG TCC CTC ACC CTC CCA AAA G-3

β-actin reverse

5-AAG CAA TGC TGT CAC CTT CCC-3

Quantitative Phytochemical Estimation Assays

Total antioxidant/Flavonoid/Phenol composition (mg/100g)

Total antioxidant

The total antioxidant activity of *Spondias mombin* essential oil was carried out using the phosphomolybdenum method by [29]. Molybdenum is reduced from (VI) to green phosphate Molybdenum (V) complex by the essential oil. Exactly 0.3 ml of the essential oil was mixed with 3 ml of (0.6 molar H_2SO_4, 28 millimolar Na_2PO_4 and 4 millimolar ammonium molybdate) in solution as reagent. The reaction undergone incubateion at 95°C for 90 minutes. This was read spectrophotometrically at 695 nm. The total antioxidant composition was expressed as mg gallic acid equivalent per 100g.

Total phenol

[30].'s method was employed. Exactly 0.5 ml essential oil was pipetted into one microliters of Fooling-Dennis reagent, one microliter of 7% $Na_2CO_3.10H_20$ and five microliters of diluting water. This was vortexed and left to stand for thirty minutes and afterwards read spectrophotomically at the appropriate wavelength. Gallic acid was used as standard. mg/100g.

Total Flavonoid Content

This was quantified by $AlCl_3$ colorimetry [31]. The isolated essential oil (1 microliter) was added to 300 µL of 5% Sodium nitrate and six hundred microliters of 6 minutes old 10% $AlCl_3$ mixture. Four microliters of 1 molar Sodium hydroxide are added to the reaction mixture, made up to 10mL. This was quantified with a spectrophotometer at the appropriate wavelength. The graph generated from the parameters (/gram) was against a solution of mg quercetin as standard.

Statistical Analysis

Statistical analysis was carried out using GraphPad Prism 5.01 Software. All experimental measurements were carried out in triplicate, eexpressed 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 post-tests or Tukey Multiple comparison test.

RESULTS

Total Antioxidant/Flavonoid/Phenol Composition

Total phenolic assay was carried out based on the absorbance values of the various extract solutions, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of pyrocatechol equivalents as described above. Data obtained from the total phenolic assay supports the key role of phenolic compounds in free radical scavenging and/or reducing systems with the essential oil the essential oil displaying total antioxidant activity of 31.75 mg/100g, total phenol

of 24.24 mg/100g and total flavonoid content of 38.28 mg/100g (Figure I).

The Wound Contraction For 14 Days

Spondias mombin essential oil ointment treatment groups have shown better wound contraction than those of the control group, even though the 1% essential oil group had a slow significant initial effect. The 10% essential oil group outperformed the standard 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.

Cytokines Expression Determination through qPCR

Although fluorogenic probes are considered more sensitive than fluorescent dyes, this study employed the use of potent SYBR Green real-time RT-PCR protocols to assay pro-inflammatory cytokines (IL1β, IL6 and TNFα) in rats. This method enables normalisation against a housekeeping gene (beta-actin) using comparative CT quantification method. PCR efficiency and sensitivity allow the assessment of; i) basal mRNA levels in many tissues and even decreases in mRNA levels, ii) mRNA levels from very small samples.

Linearity and Efficiency of PCR Amplification

The PCR curves are nice and parallel. Technical replicates are within 0.5 cycles and the CTs are <35. These are all good signs of good PCR amplification.

Cytokines Mean Plots

The graphs below show that *Spondias mombin* L. essential oil stimulated an anti-inflammatory effect through a significant reduction of IL-6 levels as compared to the vehicle and standard treatment on the 3rd, 10th and 14th day. The effect of the oil was not significant on IL1β on any sample collection day.

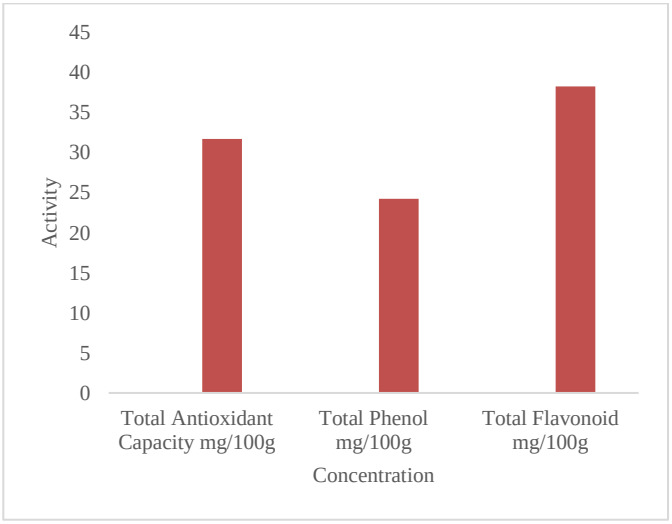

Figure 1: Graph of *Spondias mombin* essential oil Total Antioxidant/Flavonoid/Phenol composition assay showing total antioxidant activity of 31.75 mg/100g, total phenol of 24.24 mg/100g and total flavonoid content of 38.28 mg/100g

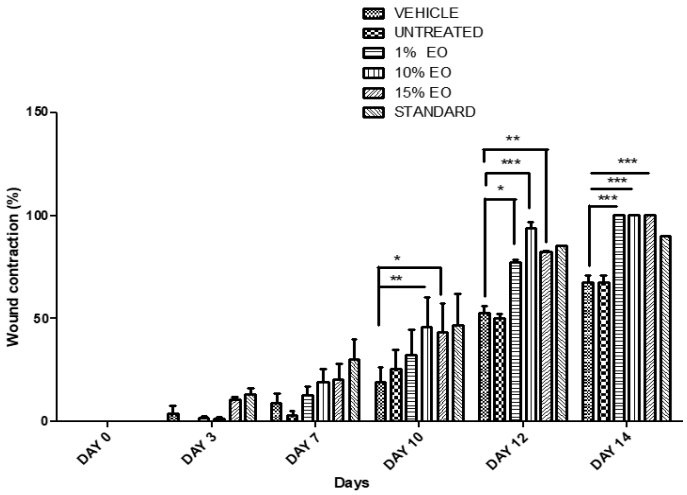

Figure 2: The mean and percentage of wound contraction for each treatment group treated with vehicle, 1% *Spondias mombin* essential oil, 10% *Spondias mombin* essential oil and the standard drug, Dermazin® after 14 days. *p<0.05 and **p<0.01 and ***p<0.001 vs vehicle/standard, using two-way ANOVA followed by Bonferroni posttests

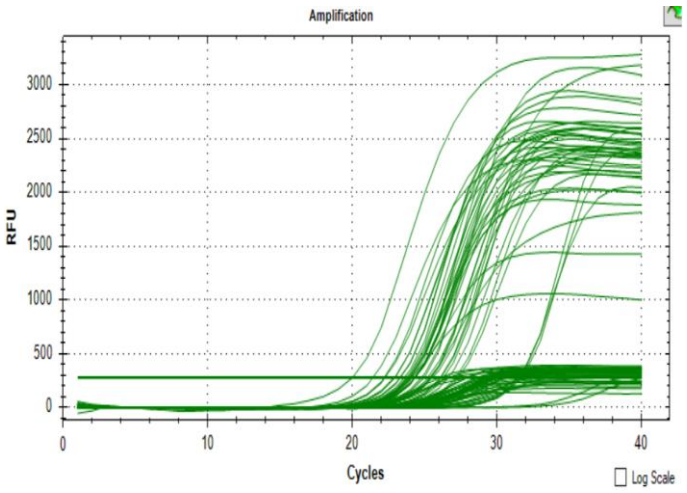

Figure 3: Showing the IL1β amplification curve

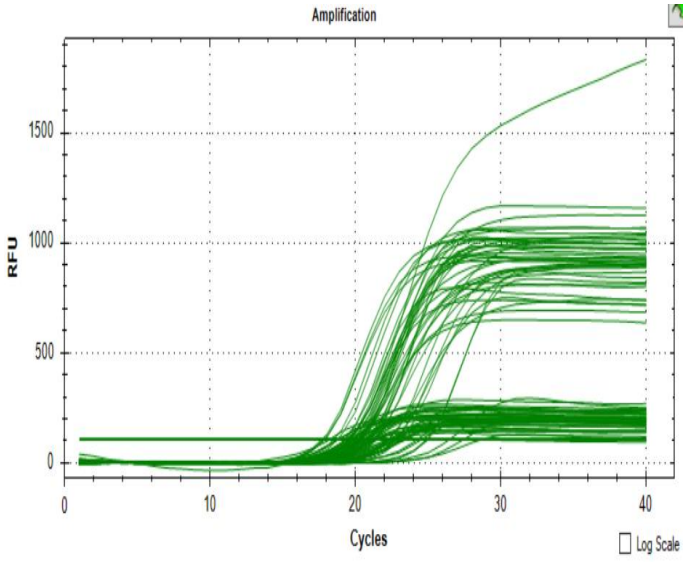

Figure 4: Showing the IL6 amplification curve

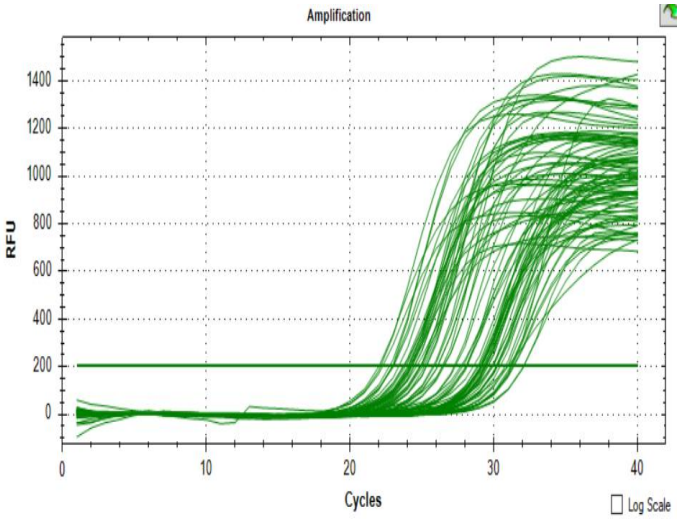

Figure 5: Showing the TNFα amplification curve

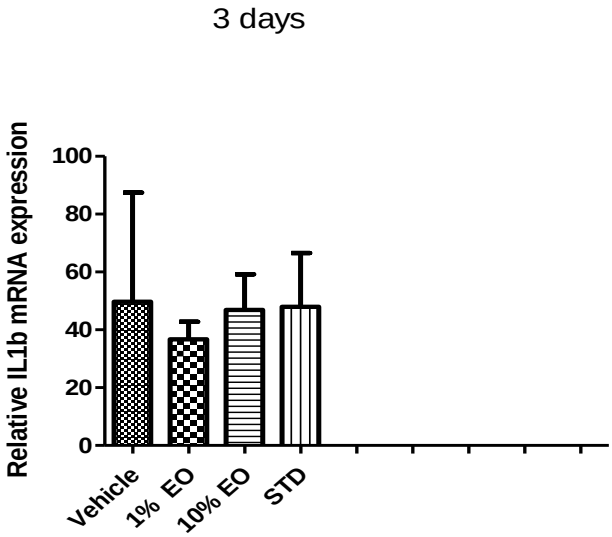

Figure 6: Showing gene expression (RT-qPCR) of IL1β in rat cutaneous lesions treated with vehicle, 1% *Spondias mombin* essential oil, 10% *Spondias mombin* essential oil and the standard drug, Dermazin® for 3 days. Using ANOVA followed by Tukey Multiple comparison test

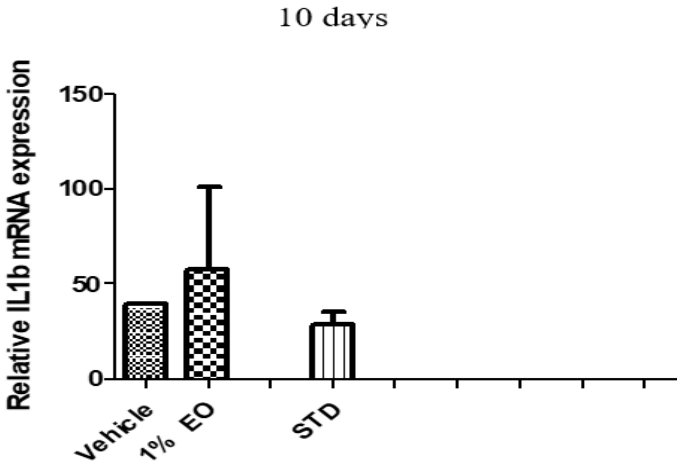

Figure 7: Showing gene expression (RT-qPCR) of IL1β in rat cutaneous lesions treated with vehicle, 1% *Spondias mombin* essential oil, 10% *Spondias mombin* essential oil and the standard drug, Dermazin® for 10 days. Using ANOVA followed by Tukey Multiple comparison test

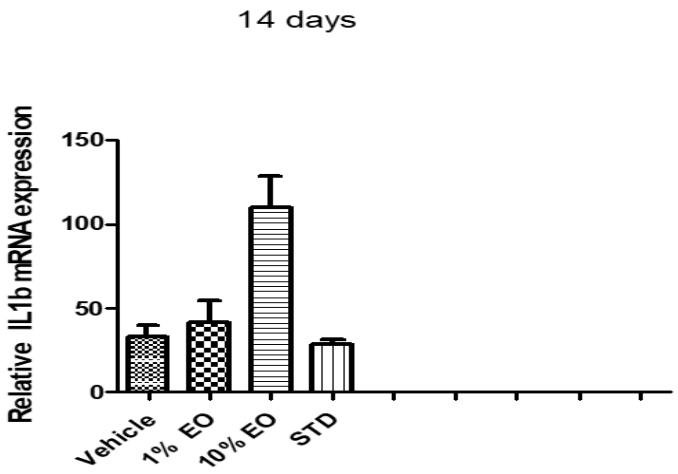

Figure 8: Showing gene expression (RT-qPCR) of IL1β in rat cutaneous lesions treated with vehicle, 1% *Spondias mombin* essential oil, 10% *Spondias mombin* essential oil and the standard drug, Dermazin® for 14 days. Using ANOVA followed by Tukey Multiple comparison test

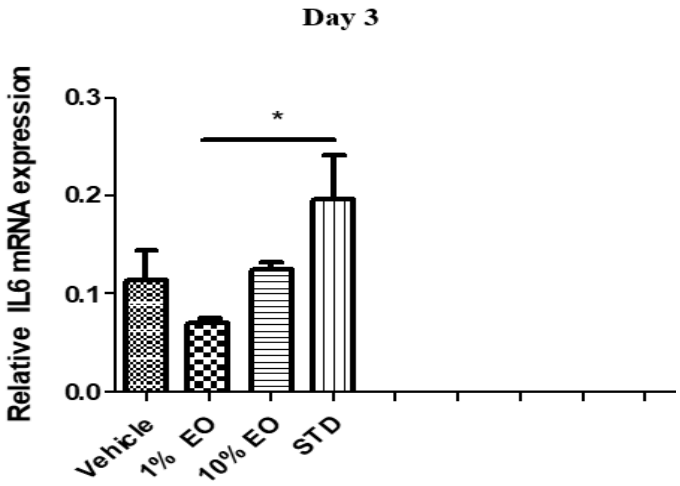

Figure 9: Showing gene expression (RT-qPCR) of IL 6 in rat cutaneous lesions treated with vehicle, 1% *Spondias mombin* essential oil, 10% *Spondias mombin* essential oil and the standard drug, Dermazin® for 3 days. *p<0.05 vs vehicle/standard, using ANOVA followed by Tukey Multiple comparison test

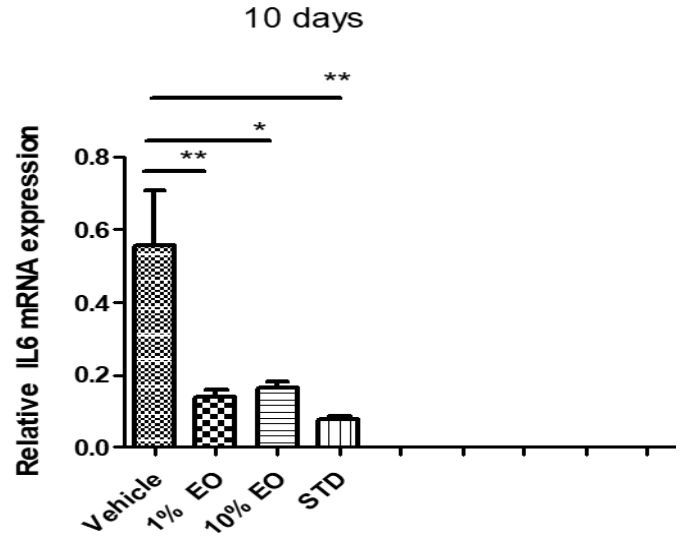

Figure 10: Showing gene expression (RT-qPCR) of IL6 in rat cutaneous lesions treated with vehicle, 1% *Spondias mombin* essential oil, 10% *Spondias mombin* essential oil and the standard drug, Dermazin® for 10 days. *p<0.05 and **p<0.01 vs vehicle/standard, using ANOVA followed by Tukey Multiple comparison test

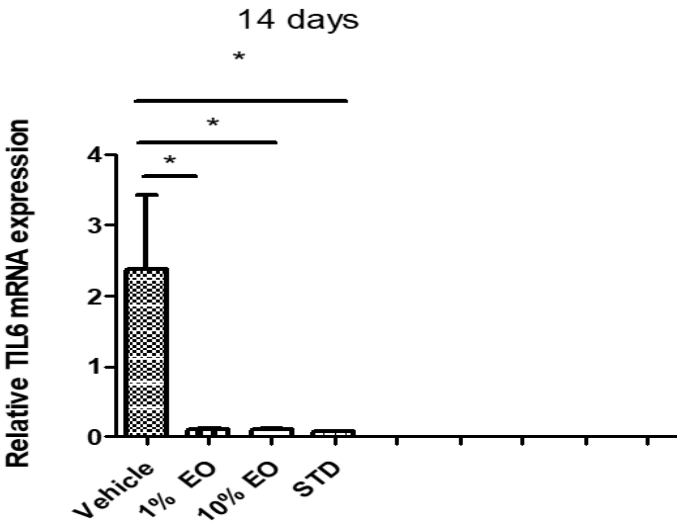

Figure 11: Showing gene expression (RT-qPCR) of IL6 in rat cutaneous lesions treated with vehicle, 1% *Spondias mombin* essential oil, 10% *Spondias mombin* essential oil and the standard drug, Dermazin® for 14 days. *p<0.05 vs vehicle/standard, using ANOVA followed by Tukey Multiple comparison test

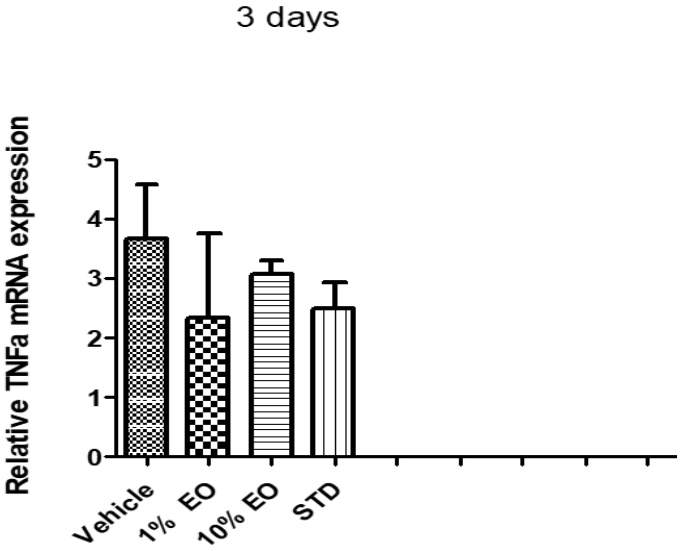

Figure 12: Showing gene expression (RT-qPCR) of TNFα in rat cutaneous lesions treated with vehicle, 1% *Spondias mombin* essential oil, 10% *Spondias mombin* essential oil and the standard drug, Dermazin for 3 days, using ANOVA followed by Tukey Multiple comparison test

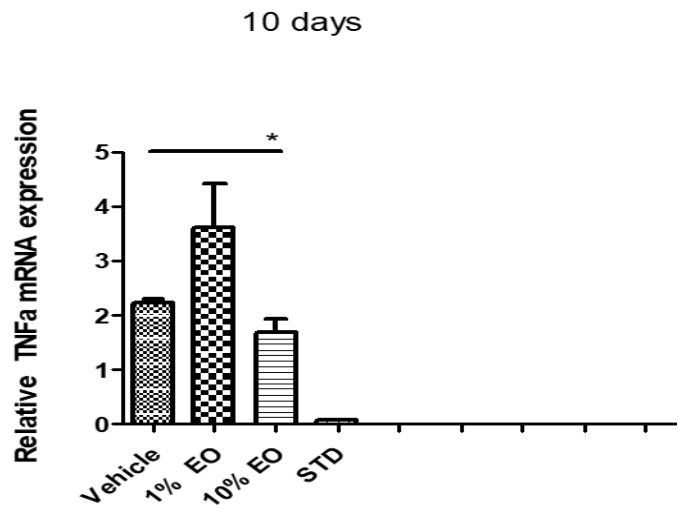

Figure 13: Showing gene expression (RT-qPCR) of TNFα in rat cutaneous lesions treated with vehicle, 1% *Spondias mombin* essential oil, 10% *Spondias mombin* essential oil and the standard drug, Dermazin® for 10 days. *p<0.05, using ANOVA followed by Tukey Multiple comparison test

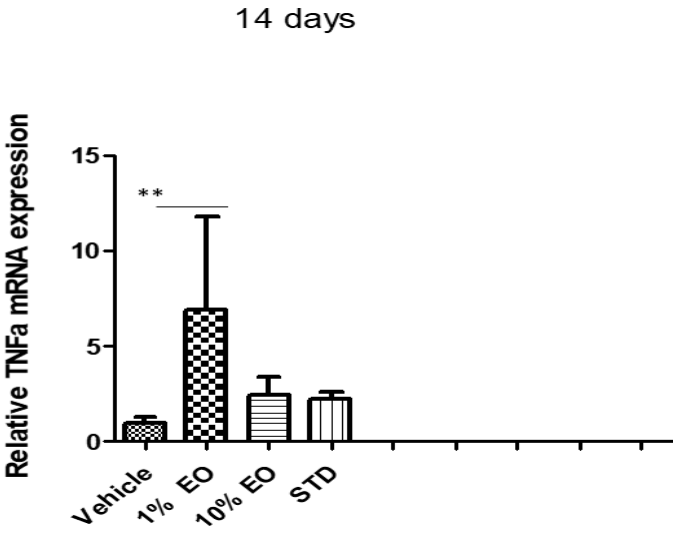

Figure 14: Showing gene expression (RT-qPCR) of TNFα in rat cutaneous lesions treated with vehicle, 1% *Spondias mombin* essential oil, 10% *Spondias mombin* essential oil and the standard drug, Dermazin® for 14 days. **p<0.01 vs vehicle/standard, using ANOVA followed by Tukey Multiple comparison test

DISCUSSION

Inflammation plays an important role in early wound healing stages enhancing proper fibroblast and collagen remodelling and arrangement [32]. The inflammatory phase must be terminated so as to proceed to the repair phase, this is a key change that must occur for proper wound healing. IL-6 is at the centre of this switch as it is involved in the signalling of fibroblasts, re-epithelialization and vascularization and subsequent differentiation of immature fibroblasts to myofibroblasts and formation of granulation during cutaneous wound healing. This can also be explained by down-regulation of IL6, because low IL6 levels stimulate maturation of fibroblasts, which promotes wound closure in the healing process. If this goes wrong, fibrosis occurs [33,34]. Therefore, the effect of *Spondias mombin* essential oil on IL6 and its subsequent effects on IL1β and TNFα production was investigated after wound treatment. This study results showed that *Spondias mombin* essential oil stimulated an anti-inflammatory effect through a significant reduction of IL-6 levels as compared to the vehicle and standard treatment on the 3rd, 10th through to the 14th day of the treatment course (Figures 21-23).

Therefore, when IL6 is controlled, proper wound healing occurs. (For example, Corticosteroids reduce IL6, VEGF, STAT3 levels to improve hypertrophic/keloid scars [35]. Tocilizumab (IL6 receptor alpha antagonist) reduces IL6, VEGF and consequently increase apoptosis of fibroblasts to improve hypertrophic/keloid scars [35]. The 10% *Spondias mombin* essential oil significantly reduced TNFα expression on the 10th day compared to the standard. (Figure 25) TNFα is regulated to lower levels during the repair process as compared to the inflammatory phase some seconds to hours after injury. There was no significant reduction in the gene expression of IL1β in the three periods of treatment.

Spondias mombin Linn. (Anacardiaceous) essential oil contains Alkaloids (inhibits arachidonic acid synthesis), phenolics, terpenes (especially Beta-Caryophyllenes, an essential oil component wwidely distributed in essential oils, a wound healing candidate which works through reduction of inflow of inflammatory cytokines by blocking of the Toll receptor and CD14 receptor [21,22]. and flavonoids which have been proven to enhance antioxidation, help reduce microorganisms on wound site and help create conditions for regeneration of fibroblasts.

[36]. Which can be linked to wound antioxidation and increase in Superoxide Dismutase enzymes to provide healing effects [37]. 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.

Some inflammatory biomarkers and phases that are postulated to be regulated by Spondias Mombin essential oil are NFκB: A transcription factor that is increased to express cytokines (IL6, IL1β and TNFα) and enzymes during wound healing conditions [38]. Ki-67: Help in the formation of granulation tissues, neo formation of blood vessels and construction of extracellular matrix [39]. Vascular Endothelial Growth Factor VEGF, a central mediator of blood vessel formation and finally, the remodeling phase: Here, proteoglycan and collagen molecules are re-arranged and remodeled in closer bundles to form high tension fibers [40]. More extensive research on gene expression and cytokines analysis to confirm the effects of *Spondias mombin* essential oil on NFκB, Ki-67, Transforming Growth Factor β TGFβ, Vascular Endothelial Growth Factor VEGF and other biomarkers needs to be done.

CONCLUSION

Based on this study, it can be said that *Spondias mombin* Linn. (Anacardiaceous) leaves essential oil contains therapeutic components that accelerates wound healing through mechanisms such as counter-inflammation, modulation of anti-inflammatory cytokines, modulating other biomarkers that help in formation of granulation tissues, neo formation of blood vessels and construction of extracellular matrix, converging activity of keratinocytes at the re-epithelization phase and tissue remodeling. Therefore, *Spondias mombin* Linn. (Anacardiaceous) essential oil is a lead for therapeutic use.

Acknowledgement

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

Authors contribution

Omiyale Olumakinde Charles: Calendar months effort, conducted the Quantitative phytochemical estimation 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

1. Abonyi DO, Abonyi MU, Esimone CO, Ibezim EC. Plants as sources of antiviral agents. Afr. j. biotechnol. 2009;8(17):3989-94.
2. Atsu Barku VY. Antioxidant and wound healing properties of some selected Plants from kpando traditional area: Isolation of flavonoids from Anogeissus Leiocarpus (dc) Guill and Perr (combretaceae) (Doctoral dissertation, University of Cape Coast). https://afribary.com/works/antioxidant-and-wound-healing-properties-of-some-selected-plants-from-kpando-traditional-area-isolation-of-flavonoids-from-anogeissus-leiocarpus-dc-guill-and-perr-combretaceae. Accessed 01 May. 2022.
3. Nworu CS, Akah PA, Okoye FB, Toukam DK, Udeh J, Esimone CO, *et al*. The leaf extract of Spondias mombin L. displays an anti-inflammatory effect and suppresses inducible formation of tumor necrosis factor-α and nitric oxide (NO). J. Immunotoxicol. 2011;8(1):10-6.
4. Onwuka CF. Hydrocyanic acid contents of tropical browse and their influence on performance of goats. Food Chemistry. 1992;45(1):5-10.
5. Fred-Jaiyesimi A, Kio A, Richard W. α-Amylase inhibitory effect of 3β-olean-12-en-3-yl (9Z)-hexadec-9-enoate isolated from Spondias mombin leaf. Food Chem. 2009;116(1):285-8.
6. Oluwatoyin AE, Deborah OW. Study of aqueous leaf extracts of Spondias mombin Linn. (Anacardiaceae) in gastric ulcer models and the possible mechanisms of action. J. Phytopharmacol. 2019;8(5):238-47.
7. Ayoka AO, Akomolafe RO, Akinsomisoye OS, Ukponmwan OE. Medicinal and economic value of Spondias mombin. Afr J Biomed Res. 2008;11(2):129–36.
8. Corthout J, Pieters L, Claeys M, Berghe DV, Vlietinck A. Antibacterial and molluscicidal phenolic acids from Spondias mombin. Planta Med. 1994;60(05):460-3.
9. Agbaje EO, Ismail AI, Oguntokun OJ. Antimicrobial and antipyretic activities of aqueous leaf extract of Spondias mombin Linn. (Anarcadiaceae). J Ethnobiol Ethnomed. 2020;6:21-9.
10. Ishola IO, Ikuomola BO, Adeyemi OO. Protective role of Spondias mombin leaf and Cola acuminata seed extracts against scopolamineinduced cognitive dysfunction. Alexandria J. Med. 2018;54(1):27-39.
11. Martins VL, Caley M, O' Toole EA. Matrix metalloproteinases and epidermal wound repair. Cell Tissue Res. 2013;351(2):255-68.
12. Kasuya A, Tokura Y. Attempts to accelerate wound healing. J. Dermatol. Sci. 2014;76(3):169-72.
13. Xia MY, Wang MW, Cui Z, Tashiro SI, Onodera S, Minami M, *et al*. Dracorhodin perchlorate induces apoptosis in HL-60 cells. Journal of Asian natural products research. 2006;8(4):335-43.
14. Roodbari N, Sotoudeh A, Jahanshahi A, Takhtfooladi MA. Healing effect of Adiantumcapillus veneris on surgical wound in rat. Res. opin. anim. vet. sci. (Online). 2012;2(12):591-5.
15. Sasidharan I, Menon AN. Comparative chemical composition and antimicrobial activity fresh & dry ginger oils (Zingiber officinale Roscoe). Int. J. Curr. Pharm. Res. 2010;2(4):40-3.
16. Tsala DE, Amadou D, Habtemariam S. Natural wound healing and bioactive natural products. Phytopharmacology. 2013;4(3):532-60.
17. Pinto ND, Cassini-Vieira P, de Souza-Fagundes EM, Barcelos LS, Castañon MC, Scio E, *et al*. Pereskia aculeata Miller leaves accelerate excisional wound healing in mice. Journal of Ethnopharmacology. 2016;194:131-36.
18. Olufunke MD, Kasali AA, Olusegun E. Constituents of the Spondias mombin Linn and the comparison between its fruit and leaf essential oils. J. Essent. Oil-Bear. Plants. 2003;6(3):148-52.
19. Ogunwande IA, Eresanya O, Oyegoke T, Ogunmoye A, Flamini G. Chemical composition of essential oils from Nigerian plants. 2012;3(1):279-86.
20. Offiah VN, Anyanwu II. Abortifacient activity of an aqueous extract of Spondias mombin leaves. J. Ethnopharmacol. 1989;26(3):317-20.
21. Koyama S, Purk A, Kaur M, Soini HA, Novotny MV, Davis K, *et al*. Beta-caryophyllene enhances wound healing through multiple routes. PloS one. 2019;14(12):e0216104.
22. Ku CM, Lin JY. Anti-inflammatory effects of 27 selected terpenoid compounds tested through modulating Th1/Th2 cytokine secretion profiles using murine primary splenocytes. Food chemistry. 2013;141(2):1104-13.
23. Gertsch J. Antiinflammatory cannabinoids in diet–towards a better understanding of CB2 receptor action? Towards a better understanding of CB2 receptor action?. Communicative & Integrative Biology. 2008;1(1):26-8.
24. Zeng Q, Xie H, Song H, Nie F, Wang J, Chen D, *et al*. In vivo wound healing activity of Abrus cantoniensis extract. Evidence-Based Complementary and Alternative Medicine. 2016;(4):1-7.
25. Pharmacopoeia B. Hmso. London. 1988;2:A137-8.
26. Jiang XW, Qiao L, Liu L, Zhang BQ, Wang XW, Han YW et al. Dracorhodin perchlorate accelerates cutaneous wound healing in wistar rats. Evidence-Based Complementary and Alternative Medicine. 2017;2017.
27. Hartman D, Coetzee JC. Two US practitioners' experience of using essential oils for wound care. Journal of Wound Care. 2002;11(8):317-20.
28. Amsen D, Visser KE, Town T. Approaches to determine expression of inflammatory cytokines. InInflammation and Cancer. 2009;(pp. 107-142).
29. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 1965;16(3):144-58.
30. Menichini F, Tundis R, Bonesi M, Loizzo MR, Conforti F, Statti G, *et al*. The influence of fruit ripening on the phytochemical content and biological activity of Capsicum chinense Jacq. cv Habanero. Food Chemistry. 2009;114(2):553-60.

31. Qian P, Song G, Xiao Z. The function of platelet endothelial cell adhesion and proinflammatory mechanism of TNF-α and IL-1. Modern Rehabilitation. 2003.
32. Nishikai-Yan Shen T, Kanazawa S, Kado M, Okada K, Luo L, Hayashi A, *et al*. Interleukin-6 stimulates Akt and p38 MAPK phosphorylation and fibroblast migration in non-diabetic but not diabetic mice. PloS one. 2017;12(5):e0178232.
33. Dunkin CS, Pleat JM, Gillespie PH, Tyler MP, Roberts AH, McGrouther DA, *et al*. Scarring occurs at a critical depth of skin injury: precise measurement in a graduated dermal scratch in human volunteers. Plastic and reconstructive surgery. 2007;119(6):1722-32.
34. Park JH, Chun JY, Lee JH. Laser-assisted topical corticosteroid delivery for the treatment of keloids. Lasers in medical science. 2017;32(3):601-8.
35. Abedini R, Sasani P, Mahmoudi HR, Nasimi M, Teymourpour A, Shadlou Z, *et al*. Comparison of intralesional verapamil versus intralesional corticosteroids in treatment of keloids and hypertrophic scars: a randomized controlled trial. Burns. 2018;44(6):1482-88.
36. Chaudhari M, Mengi S. Evaluation of phytoconstituents of Terminalia arjuna for wound healing activity in rats. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2006;20(9):799-805.
37. Ullah HM, Zaman S, Juhara F, Akter L, Tareq SM, Masum EH, *et al*. Evaluation of antinociceptive, in-vivo & in-vitro anti-inflammatory activity of ethanolic extract of Curcuma zedoaria rhizome. BMC complementary and alternative medicine. 2014;14(1):1-2.
38. Gomes MS, Lins RD, Langassner SM, da Silveira ÉJ, de Carvalho TG, de Sousa Lopes ML, *et al*. Anti-inflammatory and antioxidant activity of hydroethanolic extract of Spondias mombin leaf in an oral mucositis experimental model. Arch. Oral Biol. 2020;111:104664.
39. Brito SA, Barbosa IS, de Almeida CL, de Medeiros JW, Silva Neto JC, Rolim LA, *et al*. Evaluation of gastroprotective and ulcer healing activities of yellow mombin juice from Spondias mombin L. PloS one. 2018;13(11):e0201561.
40. Metibemu DS, Akinloye OA, Akamo AJ, Okoye JO, Ojo DA, Morifi E, *et al*. VEGFR-2 kinase domain inhibition as a scaffold for anti-angiogenesis: Validation of the anti-angiogenic effects of carotenoids from Spondias mombin in DMBA model of breast carcinoma in Wistar rats. Tox. Rep. 2021;8:489-98.

HOW TO CITE THIS ARTICLE

Agbaje EO, Charles OO. Anti-inflammatory and Cytokines Modulatory Activities of *Spondias mombin* Linn. (Anacardiaceous) in Wound Healing: Roles of IL6. J Phytopharmacol 2022; 11(4):260-267. doi: 10.31254/phyto.2022.11406