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Obi Peter Adigwe
Director General/Chief Executive Officer, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria

Bulus Adzu
Department of Pharmacology and Toxicology, NIPRD, Abuja, Nigeria

Christiana Yetunde Isimi
Department of Pharmaceutical Technology and Raw Materials Development, NIPRD, Abuja, Nigeria

Jemilat Aliyu Ibrahim
Department of Medicinal Plants Research and Traditional Medicine, NIPRD, Abuja, Nigeria

Samuel Ehiabhi Okhale
Department of Medicinal Plants Research and Traditional Medicine, NIPRD, Abuja, Nigeria

Aliyu Usman
Department of Pure and Applied Chemistry, Adamawa State University, Mubi, Nigeria

Corresponding Author:
Obi Peter Adigwe
Director General/Chief Executive Officer, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria

Essential oils from *Sphaeranthus senegalensis* DC accelerate excision wound healing

Obi Peter Adigwe, Bulus Adzu, Christiana Yetunde Isimi, Jemilat Aliyu Ibrahim, Samuel Ehiabhi Okhale and Aliyu Usman

Abstract
Sphaeranthus senegalensis DC is an aromatic annual herb that grows after the season in damp and swampy areas of tropical Africa and Asia. The plant is used for various medicinal uses that include sedative, anti-inflammatory, anti-nociceptive, antidiarrhoeal and gastroprotective. This study focuses on the essential oils from the plant with the aim of evaluating its possible therapeutic usefulness. The fresh plant material was obtained and subjected to hydrodistillation and solvent extraction methods. The chemical constituents of the oils obtained were identified using GC-MS techniques. Hydrocarbons were prevalent, totalling 53.77% in the oil obtained through hydrodistillation and 80.95% for the oil obtained through solvent extraction. Oxygenated constituents constituted 43.76% and 19.15%, respectively. The oil obtained through solvent extraction was tested for acute dermal toxicity and wound-healing effect in rats. Data obtained showed that the oil is safe on the skin and exhibited wound healing activity.

Keywords *Sphaeranthus senegalensis*, essential oils, GC-MS, dermal toxicity, wound healing

1. Introduction
The skin functions as a barrier that protects the body from external environment, act as pathogens and thermal barrier and prevents dehydration. Wound is an injury in which the skin is cut or broken; and so requires immediate recovery in order to avoid further complications and pathogenesis [1, 2]. The prevalence of wounds is believed to affect approximately 1% of the population worldwide resulting mainly in situations of natural and manmade calamities [3]. In Nigeria for example, wounds constitute about 30–42% of hospital attendance and 9% death every year, with higher incidence in males than females [4, 5]. Wound healing restores the skin anatomical and functional integrity, and is a complex method that begins at the time an injury occurs and goes on for certain duration based on the level of the injury [6]. These events are sequence of interactions between molecules and cells resulting in inflammation, re-epithelization, tissue formation and remodelling [7-9]. Time is an important aspect in the wound healing process, and researchers have been looking for new products and therapies that can accelerate the process and give better healed skin quality [10, 11].
Despite therapeutic successes being made with existing wound healing medications, topical agents are still sought after, especially from natural sources, to expand the therapeutic arsenal and the possibility of discovering more effective agents [12]. Essential oils are one of the main causes of the pharmaceutical properties of plants, providing vast pharmacological active ingredients in just a single drop of the oil. They serve as a source of drugs from natural products and evidences shows they great influence in wound healing [13-15].
Sphaeranthus senegalensis DC (family, Compositae) is an aromatic annual herb that grows at the beginning of dry season in damp and swampy area of tropical Africa and Asia. The medicinal uses of the plant and its description, as well as its various biological activities that include sedative, anti-inflammatory, anti-nociceptive, antidiarrhoeal and antiulcer, are well documented [16-20]. This study aimed at investigating the plant's essential oils; by evaluating its chemical constituents, dermal safety and wound healing activity against excision wound model.

2. Materials and Methods
2.1 Plant material
Sphaeranthus senegalensis DC., Prodr. [A.P. de Candolle] 5: 370 (1836) (http://www.ipni.org) was collected in Mubi, Adamawa State, Nigeria (10°16′N 13°16′E) in December 2020. It was identified by a botanist Lateef Akeem Adeyanju and checked at www.theplantlist.org.

A voucher specimen (NIPRD/H/7238) is kept at the NIPRD's Herbarium.

2.2 Extraction of essential oils

2.1.1 Hydrodistillation

The fresh whole plant of *Sphaeranthus senegalensis* was cleaned, chopped into pieces, and (300 g) immersed in 1 L of distilled water using a Clevenger – type apparatus of 2 L capacity. It was then heated on a mantle continuously for 4 h, in which the azeotropic mixtures of the volatile components are carried by steam to a condenser at the front of the receiver. This was then collected, stored in sealed vials, and analyzed within 30 min [21].

2.2.2 Solvent extraction

In this procedure, fresh plant material was pounded into a paste, weighed, and placed in 1 L round bottom flask. It was extracted in multiples of 100 g each with petroleum ether (1:10 w/v) for 72 h with occasional shake twice a day. The mixtures were then filtered through a filter paper, and the filtrates were concentrated in an oven maintained at 40 ^{O}C, then allowed to cool. The crude oil extracts obtained were purified by passing it through a column of silica gel, eluting with n-hexane: ethyl acetate at a ratio of 10:1; to obtained essential oils from *Sphaeranthus senegalensis* (denoted EOSs) and the recovery, yield calculated [22].

2.3 Moisture content

This was determined using the Karl Fischer titration instrument (ZDY-502 Karl Fischer Titrator, Shanghai INESA Scientific Instrument Co., China).The procedure measures the water content of a sample selectively by means of a chemical reaction:

$H_2O + SO_2 + I_2 + MeOH + 3C_5H_5N \rightarrow 3\ C_5H_5NH^+ + 2I^- + MeSO_4^-$ [23, 24]. The sample was placed in a vapour tight titration cell and was titrated using Karl Fischer (KF) reagent (the all-in-one component containing sulphur dioxide, pyridine, iodine and methanol) under the following condition: Real time, 00:02:54; stirrer, 1 speed; burette, 10 mL; Factor, 100%; adding volume, 0.02 mL; sample quantity, 67.95 mg; and KF titre, 5 mg/mL. This reagent was first calibrated versus water-methanol mixture standard.

2.4 GC-MS analysis

The essential oils obtained from both techniques were analyzed by GC-MS. The chromatographic conditions used are stated in Table 2:

Table 1: The chromatographic conditions used are stated

Item	Specification
Instrument	Shimadzu®QP-2010 GC, Kyoto, Japan
Detector	QP-2010 mass selective detector [MSD]
Electron ionization (EI) mode	Electron energy = 70 eV
Scan range	45-400 amu
Scan rate	3.99 scans/sec
Column	Optima – 5 m fused silica capillary with: a 5% phenyl-methylpolysiloxane stationary phase; length = 30 m; internal diameter = 0.25 mm, and film thickness = 0.25 µm
Carrier gas / flow rate	Helium / 1.6 mL/min
Mode/flow rate	Linear gradient/0.6 mL/min
Oven temperature program	60 – 180°C at a rate of 10°C/min, then held at 180°C for 2 min; 180 – 280°C at a rate of 15°C/min; and then 280°C for 4 min
Injection port temperature	250°C
Detector temperature	280°C
Split mode ratio	10:90

The sample was diluted in hexane (1/100, v/v), out of which 1 µL was injected using an autosampler. For all the volatile constituents, retention indices were calculated, and individual constituents were identified by comparing their retention time with literature data.

2.5 Animal care and ethics

Wistar rats (*Rattus norvegicus*) were obtained from the Animal Facility Centre (AFC) of NIPRD, Abuja. The animals were used in line with NIPRD Animal Care and Ethic's (NIPRD/05:3:05–13), as required by the International Guiding Principles for Biomedical Research Involving Animals [24].

2.6 Pharmacological evaluations

2.6.1 Acute dermal toxicity test

The OECD [25] Test Guideline 402 was adopted for the test. Briefly, rats were acclimatized for 5 days, after which hairs on the dorsal part of the animals (n = 3) were removed 24 h before the test. EOSs was evenly applied to cover the exposed area, covered with porous non-adhesive gauze, and placed individually in clean cages. The oil residue was carefully removed with cotton wool after an exposure period of 24 h. The skins were observed for signs of erythema, eschar, and edema. The irritation score (erythema + edema) were taken 30 min after oil application, then hourly for 6 h, after which it was taken daily for 14 days. Observations were recorded using the Draize skin test scoring system as follows: very slight (barely perceptible), 1; well-defined, 2; moderate to severe, 3; and severe injuries, 4. Changes in eyes and mucous membrane, skin, and fur, respiratory, central nervous systems, as well as salivation, diarrhea, and changes in weight, motor, and behavioral activities, were all noted.

2.6.2 Wound healing activity

The excision wound healing model [26, 27] was adopted with little modification. Rats were weighed and grouped into three (n = 6), restrained ventral decubitus on OP-Table (Hugo Sachs Elektronic, D-79232, Grünstraße 1, Germany) and injected intradermal with local anaesthesia (0.05 mL of 0.5% lidocaine) at the dorsal area where the excision was to be carried out. A measured area of the anaesthetized skin (1 cm by 1 cm) was removed with surgical scissors to expose

muscle fascia. The wounds were pre-cleaned with 0.9% saline and then treated topically (O.D.) with distilled water, EOSs (100%), and 1% Povidone-iodine (PVP-I) using a quantity sufficient enough to cover the injured area, daily for 14 days. Pictorial and physical measurements (using digital vernier caliper) of the wound were made on days 0, 3, 7, 14, and 21 days and the rate of wound contraction were calculated using the expression [28]:

$$\text{Rate of contraction} = \frac{\text{Area on day } 0 - \text{Area on day evaluated}}{\text{Area on day } 0} \times 100$$

The re-epithelialization period, being a number of days for the complete closure of the wounds [12], was also noted.

2.7 Statistical analysis

The results of the biological studies were expressed as mean ± standard error of the mean. Parametric one way-analysis of variance (ANOVA) was used to compare the means between groups, followed by the Student-Newman-Keuls test for multiple comparisons using Graph Pad Prism Version 5.01 software for Windows (San Diego, California, USA).

3. Results

3.1 Physicochemical parameters

The following parameters were recorded: yield, 0.5%; and moisture content, 0.146%.

3.2 Hydrodistillation

The GC-MS chromatogramsof essential oils extracted from *Sphaeranthus senegalensis* DC using hydrodistillation are shown in Figure 1.

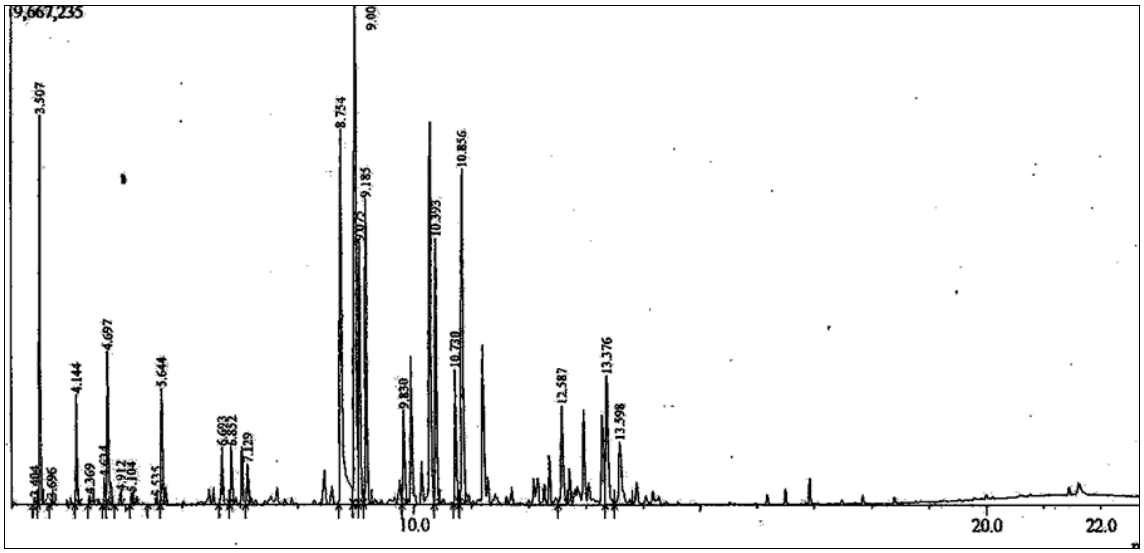


Fig 1: Chromatogram of oil components obtained through hydrodistillation showing twenty-five peaks corresponding to chemical constituents listed in Table 2

3.3 Solvent extraction

The GC-MS chromatograms of essential oils extracted from *Sphaeranthus senegalensis* DC (EOSs) using petroleum ether and purified with n-hexane: ethyl acetate is shown in Figure 2.

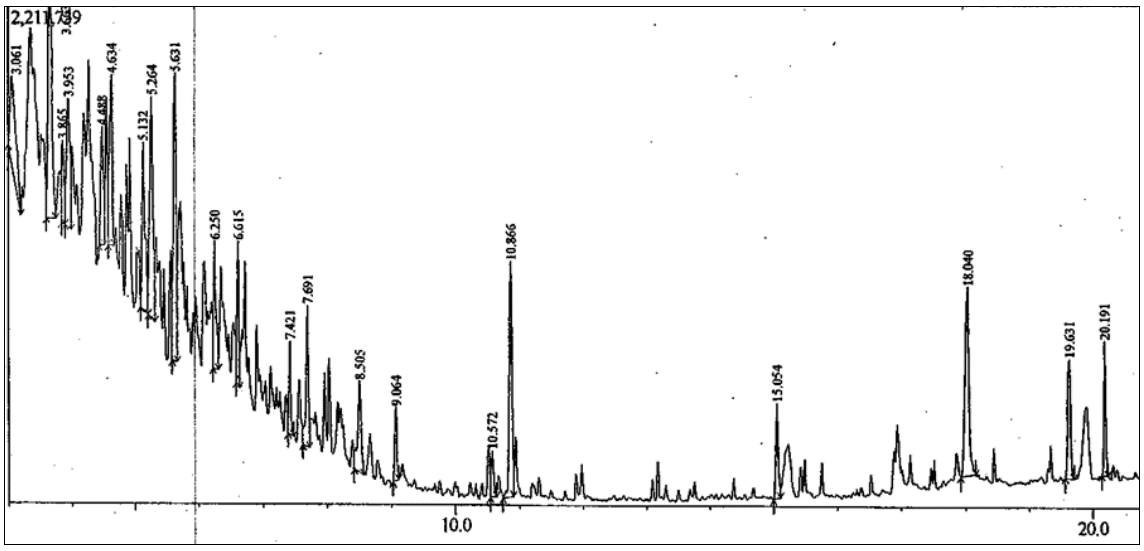
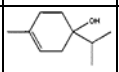
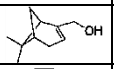
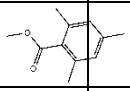


Fig 2: Chromatogram of EOSs obtained through solvent extraction showing twenty-one peaks corresponding to chemical constituents listed in Table 3

Table 2: Chemical constituents of oil from *Sphaeranthus senegalensis* obtained through hydrodistillation detected using GC-MS

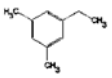
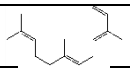
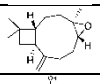
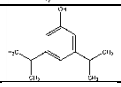
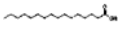
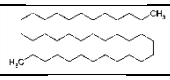
S/No	Formula	Common name	Chemical name	Structure	MW ($gmol^{-1}$)	RT (Min)	Area (%)
1	$C_{10}H_{16}$	α-thujene	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-		136.23	3.404	0.11
2	$C_{10}H_{16}$	α-pinene	-		136.23	3.507	7.80
3	$C_{10}H_{16}$	Camphene	2,2-Dimethyl-3-methylenenorbornane		136.23	3.696	0.08
4	$C_{10}H_{16}$	ß-myrcene	7-Methyl-3-methylene-1,6-octadiene		136.23	4.144	2.10
5	$C_{10}H_{16}$	α-phellandrene	-		136.23	4.369	0.17
6	$C_{10}H_{14}$	o-Propyltoluene	1-Methyl-2-isopropylbenzene		136.23	4.634	0.49
7	$C_{10}H_{16}$	D-limonene	-		134.22	4.697	3.51
8	$C_{10}H_{16}$	ß-cis-ocimene	(Z)-3,7-Dimethylocta-1,3,6,-triene		136.23	4.912	0.29
9	$C_{10}H_{16}$	Ɣ-terpinene	p-Mentha-1,4-diene		136.23	5.104	0.21
10	$C_{10}H_{16}$	Terpinolene	-		136.23	5.535	0.13
11	$C_{10}H_{18}O$	Linalool	3,7-Dimethyl-1,6-octadien-3-ol		154.25	5.644	2.43
12	$C_{10}H_{18}O$	Borneol	1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol		154.25	6.693	1.44
13	$C_{10}H_{18}O$	4-Terpineol	1-Terpinen-4-ol		154.25	6.852	1.21
14	$C_{10}H_{16}O$	Myrtenol	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-		152.23	7.129	1.06
15	$C_{11}H_{14}$	Cyclopentylbenzene	-		146.23	8.754	14.11
16	$C_{11}H_{14}O_2$	Methyl mesitoate	Methyl 2,4,6-trimethylbenzoate		178.23	9.007	17.40
17	$C_9H_{16}O_2$	-	Isopropyl (2E)-2-methyl-2-pentanoate		156.22	9.075	6.40
18	$C_{11}H_{14}O_2$	-	Methyl 3,4,5-trimethylbenzoate		226.23	9.185	7.63
19	$C_9H_{10}N_2$	-	3,3-Dimethyl-3H-indazole		146.19	9.830	2.48
20	$C_{15}H_{24}$	Caryophyllene	-		204.36	10.393	6.61
21	$C_{15}H_{24}$	ß-farnesene	-		204.36	10.730	3.09
22	$C_{15}H_{24}$	Humulene	-		204.35	10.856	10.22
23	$C_{15}H_{24}O$	Caryophyllene oxide	-		220.35	12.587	3.20
24	$C_{15}H_{24}$	Ɣ-cadinene	-		204.35	13.376	4.85
25	$C_{15}H_{26}O$	Cubenol	1-Isopropyl-4,7-dimethyl-1,3,4,5,6,8a-hexahydro-4a(2H)-naphthalenol		222.37	13.598	2.99

MW (molecular weight); RT (retention time).

GC-MS analysis identified twenty-five compounds in essential oils from S*phaeranthus senegalensis* obtained through hydrodistillation. Nine of the components (14.4%) were isomers of $C_{10}H_{16}$, with α-pinene constituting 7.80%; 3

of the components (5.08%) were isomers of $C_{10}H_{18}O$; and 4 components (24.77%) were isomers of $C_{15}H_{24}$, with humulene constituting 10.22%. Out of the compounds identified, hydrocarbons were the most prevalent, totaling fifteen compounds (53.77%); nine were oxygenated hydrocarbons (43.76%); and one nitrogen-containing compound (3,3-Dimethyl-3H-indazole, 2.48%).

Table 3: Chemical constituents of oil from *Sphaeranthus senegalensis* obtained through solvent extraction detected using GC-MS

S/No	Formula	Common name	Chemical name	Structure	MW ($gmol^{-1}$)	RT (Min)	Area (%)
1	$C_8H_{12}O_2$	Dimedone	5,5-Dimethylcyclohexane-1,3-dione		140.18	3.061	5.78
2	C_9H_{12}	Pseudocumene	1,2,4-Trimethylbenzene		120.19	3.660	10.01
3	$C_{10}H_{22}$	-	2, 3-Dimethyloctane		142.28	3.865	2.21
4	C_9H_{12}	Hemimellitene	1,2,3-trimethylbenzene		120.20	3.953	5.57
5	$C_{14}H_{22}$	-	1-Ethyl-3,5-dimethylbenzene		190.33	4.488	3.94
6	$C_{11}H_{24}$	Undecane	$CH_3(CH_2)_9CH_3$		156.31	4.634	5.16
7	$C_{10}H_{12}$	5-Methylindan	5-methyl-2,3-dihydro-1H-indene		132.20	5.132	5.98
8	$C_{10}H_{12}$	O-Allyltoluene	1-Methyl-2-(2-propenyl)benzene		132.21	5.264	8.63
9	$C_{12}H_{16}$	-	1-Ethyl-1-methylindan		160.23	5.631	9.36
10	$C_{11}H_{14}$	-	4,7-dimethyl-2,3-dihydro-1H-indene		146.23	6.250	3.41
11	$C_{13}H_{28}$	Tridecane	-		184.37	6.615	2.82
12	$C_{15}H_{24}$	Farnesene	-		204.36	7.421	1.88
13	$C_{14}H_{30}$	Tetradecane	-		198.39	7.691	3.79
14	$C_{16}H_{34}$	Hexadecane	-		226.45	8.508	3.36
15	$C_{15}H_{32}$	Pentadecane	-		212.42	9.064	1.76
16	$C_{15}H_{24}O$	Caryophyllene oxide	-		220.35	10.572	1.25
17	$C_{12}H_{18}O$	-	3, 5-Diisopropylphenol		178.28	10.886	7.20
18	$C_{16}H_{32}O_2$	Palmitic acid	Hexadecanoic acid		256.43	15.123	2.68
19	$C_{24}H_{50}$	Tetracosane			338.66	18.040	8.54
20	$C_{36}H_{74}$	Hexatriacontane			506.99	19.631	3.93
21	$C_{30}H_{62}$	Triacontane			422.83	20.191	2.83

MW (molecular weight); RT (retention time).

GC-MS analysis identified twenty-one compounds in essential oils from S*phaeranthus senegalensis* (EOSs) obtained through solvent extraction. The major constituents were pseudocumene (10.01%), 1-Ethyl-1-methylindan (9.36%), O-Allyltoluene (8.63%), tetracosane (8.54%) and 3, 5-Diisopropylphenol (7.20%). Caryophyllene oxide,

pentadecane, and farnesene were present in a low quantity of 1.25%, 1.76%, and 1.88%, respectively. EOSs were primarily (80.95%) made up of hydrocarbons, which consisted of seventeen compounds, alongside 4 oxygenated constituents (19.15%), with 3,5-diisopropyl phenol being the most abundant (7.20%).

3.4 Acute dermal Toxicity

The EOSs did not show signs of irritation reaction as defined by erythema, eschar, and edema on the skin of the treated rats. The rate of hair growth was not affected, and the general behavioral pattern of treated animals was similar to the control groups. Also, no mortality was recorded in all the animals throughout the 14-day observation period.

3.5 Wound healing effect

The effect of the treatments with EOSs and PVP-I on skin excisional wounds in rats are shown in Table 4. The wound healing started on the 3rd-day treatment with 13.22% and 13.36% for EOSs and PVP-I. Treatment of the wound area with EOSs and posidone reached a maximum effect on the 16 and 15thday with 95% and 98% ($p< 0.01$) wound contraction rate, respectively. The pictorial sample representation is presented in Figure 3.

Table 4: Effect of topical application of essential oils from *Sphaeranthus senegalensis* (EOSs) on wound contraction rate (%) and re-epithelialization period (in days) on excision wound model in rats

Treatment	Wound contraction rate (%)					
	D_3	D_7	D_{10}	D_{14}	D_{21}	RE (days)
Control	8.74±0.18	16.65±0.28	24.82±0.47	45.87±0.17	75.54±0.27	21
EOSs	13.22±0.27	31.49±0.30*	65.42±0.16*	80.91±0.14**	94.91±0.05**	16
PVP-I	13.36±0.17	36.30±0.23*	74.91±0.23*	91.48±0.19**	97.95±0.04**	15

The values are mean ± SEM (n = 6). One-way ANOVA followed by the Student-Newman-Keuls test * $p< 0.05$; **$p< 0.01$ versus Control. RE: reepithelialization period (in days).

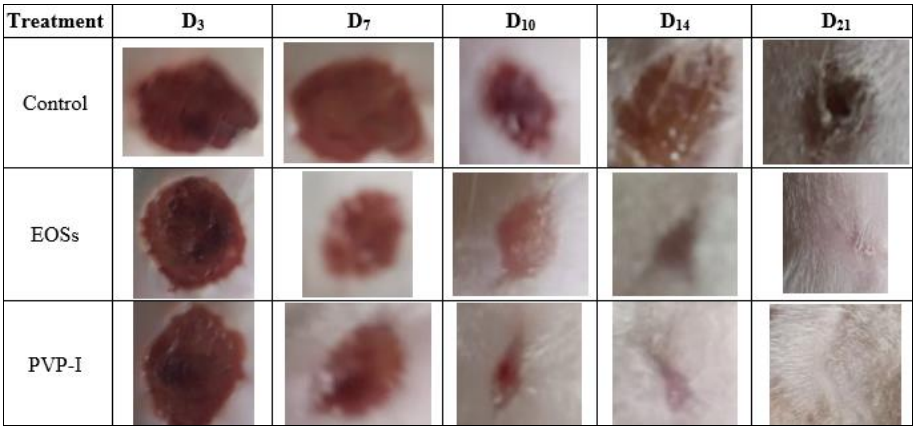


Fig 3: Macroscopic measurements of excision wound areas at 3rd 7th, 10th, 14th and 21st days

4. Discussion

Essential oils have very high pharmaceutical value due to their properties. Their mode of extraction can be achieved by various methods; chosen according to the purpose of use, and the extraction product can vary in quality, quantity, and composition. To obtain essential oils of constant composition, therefore, it has to come from the same organ of the plant, growing on the same soil, under the same climate, picked in the same season, and extracted under the same conditions. This can then be chemotyped by gas chromatography and mass spectrometry (GC-MS) analysis, making them unique for quality control and validity checks.

The analysis of chemical constituents of essential oils from *Sphaeranthus senegalensis* obtained through hydrodistillation revealed 25 components. Four of the constituents (α-pinene, isopropyl (2E)-2-methyl-2-pentanoate and methyl 3, 4, 5-trimethylbenzoate and caryophyllene) each making 5 – 10% of the constituents, while 3 of the components (cyclopentylbenzene, methyl mesitoate, and humulene) each constituting above 10% of the components. This indicates that the 7 constituents made up 70.17% of the oil components, 38.74% hydrocarbons, and 31.43% oxygenated constituents. As seen from Table 2, hydro-distilled material significantly formed a larger proportion of the lower boiling point hydrocarbons and oxygenated terpenes. These hydro-distilled constituents were therefore used as markers for chemical fingerprints profiling of *Sphaeranthus senegalensis*.

The chemical profile of the essential oil from *Sphaeranthus senegalensis* obtained by solvent extraction is quite different from that of the hydrodistillation. The data obtained shows that over 80% of constituents were hydrocarbons with pseudocumene, an aromatic hydrocarbon used as a sterilizing agent constituting 10.01%. Other major components were 1-Ethyl-1-methylindan (9.36%), O-Allyltoluene (8.63%), and tetracosane (8.54%). The main oxygenated constituent was 3, 5-Diisopropylphenol (7.20%). The only common constituent between the essential oils from *Sphaeranthus senegalensis* obtained from the hydrodistillation and solvent extraction techniques is caryophyllene oxide. A bicyclic sesquiterpene, caryophyllene oxide, is a metabolite of β-caryophyllene, a natural bicyclic sesquiterpene [29]. Such differences in the chemical constituents of the oils could be due to lower volatility of the compounds not effectively extracted by hydrodistillation and might escape the solvent extraction technique [22].

Essentials oils from organic solvent extraction are complex mixture of various aromatic chemicals. Each of these constituents contributes to the beneficial or adverse effects of the oil, thereby presenting a large spectrum of biological activities [30]. This presents the need for scientific

investigations into their novel sources for the possible discovery of more potent agents and establishes both their safety and efficacy; in order to enhance their therapeutic benefits.

Essential oils had found application in wound healing of a variety of wounds [31] and could also cause harm that includes allergic reactions and skin irritation [32]. We thus investigated the acute dermal toxicity and potential wound healing effect of EOSs. The acute dermal toxicity data shows that EOSs did not cause any change in skin color, and the rate of hair growth was comparable to the control group. There was also no abnormal behaviour recorded in any animal, indicating that EOSs are safe on the skin.

The excision wound model is the most utilized in evaluating substances with potential wound-healing effects. This is because the model evaluates the evolution of the wound healing process as determined by wound contraction rate and re-epithelialization of the skin [28]. The model is also close to some types of clinical wounds in which healing is brought by re-epithelialization, dermal reconstitution, and contraction. EOSs increased the rate of wound closure, a basic criterion of wound healing agent [12, 33]. This thus shows EOSs as a potential natural agent to repair damaged skin tissues plausibly due to its chemical constituents acting in moiety.

5. Conclusion

This study indicates that essential oils from *Sphaeranthus senegalensis* DC contain useful chemical constituents, safe on the skin with potential wound healing effects. The mechanisms of its wound-healing effect need, however, to be studied further.

6. Author contributions

The project conception was by OPA, BA designed the experimental scheme and analysed the data, CYI handled the formulation, JAI and SEO generated the GC-MS spectra, AU collected the plant material and obtained the crude extract. All authors prepared and approved the manuscript.

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8. Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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