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Botanical Evaluation, GC-MS Analysis and Anti-Inflammatory Properties of the Leaves of *Lasimorpha senegalensis* Schott (Araceae)

Felix I. Nwafor[1,2], Eleje Okonta[2]*, Helen Udodeme[2], Chima Ugorji[3], Stella Inya-Agha[2], Uchenna E. Odoh[2]

[1]*Department of Plant Science and Biotechnology, University of Nigeria, Nsukka*
[2]*Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka*
[3]*Department of Science Laboratory Technology, University of Nigeria, Nsukka*

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ABSTRACT

The leaf of *Lasimorpha senegalensis* Schott. (Araceae) is widely used as one of the traditional remedies for swellings, edema, pain, tumours and other inflammatory problems in southeastern Nigeria. However, its anti-inflammatory potential has not been scientifically established. This work evaluated the anti-inflammatory activity of its methanol leaf extract using the carrageenan-induced hind paw edema test and the cotton-pellet granuloma assay. For each model, different concentrations (200 mg/kg, 400 mg/kg and 800 mg/kg) of the extract was administered to each experimental group of Wistar rats (120 – 150 g; n=5). Ibuprofen (100 mg/kg) served as standard drug while normal saline (1 ml/kg) was used as negative control. Microscopic evaluation, quality standard and GC-MS analysis of the extract were also established following standard protocols. The extract (at all doses tested) significantly ($p < 0.05$, $p < 0.01$) decreased paw edema within the first 8 hours of treatment, to as much as 80 %, with better activity than ibuprofen. The extract also generally inhibited granuloma tissue formation (by as much as 70 %) in the rats, and in a dose-dependent manner. Microscopy revealed features of taxonomic importance such as the stomata parameters while the quality standards (ash and extractive values) were found to conform to existing monographs. Among 18 compounds identified by GC-MS, terpene-4-ol, palmitoleic acid, n-hexadecanoic acid, octadecanoic acid and 22-stigmasten-3-one are proven anti-inflammatory agents. These results justify the local use of the leaf of *L. senegalensis*, in the treatment of inflammatory conditions.

***Keywords*:** Inflammation, Carrageenan, Edema, Cotton-pellet granuloma, *Lasimorpha senegalensis*

Introduction

Inflammation is one of the common complaints, after pain, in most patients suffering from disease conditions.[1] Inflammation is a host defense mechanism to combat or overcome the invading pathogen or the foreign particles. Understanding inflammation has always been an enigma for mankind. Something as minor as a bruise or something as major as a myocardial infarction can trigger this phenomenon.[1] The major classes of drugs to suppress inflammation are nonsteroidal anti-inflammatory agents (NSAIDS) and corticosteroids but their toxic adverse effects such as epigastric distress, peptic ulceration, osteoporosis, and iatrogenic Cushing's syndrome have limited their use. In order to minimize these side effects, medicinal plants provide excellent alternative forms of healthcare delivery. Their bioactive compounds such as flavonoids, saponins, alkaloids, terpenoids, glycosides,
and coumarins remain the future building blocks in developing new anti-inflammatory agents, which could be more efficacious, safer, affordable, and accessible for patients.[2, 3] Contribution of herbal medicine (drug from botanical sources) in traditional healthcare delivery cannot be overemphasized.

***Corresponding author. E mail: eleje.okonta@unn.edu.ng
Tel: +2348068405223

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It is estimated that 80% of the world's population make use of herbal remedies, in one form or the other, to manage and/or treat ailments. Furthermore, an appreciable number (about 25%) of modern drugs commonly prescribed today as well as some other pharmaceutical products have at least one ingredient in them derived from a medicinal plant.[4, 5] Herbal drugs have gained popularity in global markets where they are sold in raw or processed forms and as formulations. However, their increased demand rate has led to concerning issues of substitution and adulteration.[6] It is to this effect that evaluation and standardization of herbal drugs came into practice. Standardization is achieved by following certain laid-down procedures that ensure quality control and reproducibility of herbal products. In this chapter, the authors highlight the various concepts of substitution/adulteration of herbal drugs, the needs for standardization, and various evaluation and analytical techniques (botanical, microscopic, physical, chemical, toxicological and biological) that ensure correct identity, purity, quality, potency, efficacy and safety of herbal drugs.[7]

Lasimorpha senegalensis Schott. (Araceae), commonly known as 'swamp arum' is a herbaceous, monocotyledonous and monospecific plant that belongs to Araceae. It remains the only African representative of the genus.[8, 9] The plant is found mainly in aquatic and/or moist habitat, where it spreads profusely through thick stoloniferous rhizhomes that produce bunch of leaves from underground, forming large populations without major biodiversity threat.[10, 11] Even though members of this family are considered toxic and irritating, mainly as a result of abundant proteinaceous toxins and calcium oxalates in their cell sap,[12] different morphological parts are wildly harvested for food and medicine. For instance, cooked leaves are taken by pregnant women in Congo to aid child delivery, and also infused as herbal concoctions for treatment of cough, agitation, nervousness and ulcer.[11, 13] The leaf sap and fruits are some of the herbal ingredients used to treat hiccups

and sexually transmitted diseases in Côte d'Ivoire and southern Nigeria respectively.[13] Cooking salt is extracted from burnt aerial parts in Sierra Leone and Gabon, while the broad leaves are used as food wrappers.[11] Ethnobotanical survey within southeastern Nigeria (unpublished) revealed that leaf decoction is taken as a traditional remedy for hepatitis and fever. Despite all these ethnomedicinal uses, there remains scarcity of research reports on its phytochemical constituents and pharmacological activities. Chigor *et al.*[14] however, reported its antioxidant and hepatoprotective potentials. Therefore, the aim of this work was to establish the its pharmacognostic and chemical profile as well as assess the potentials of the methanol leaf extract as an anti-inflammatory agent on animal models.

Materials and Methods

Plant Collection and Identification: Fresh and healthy leaves of *Lasimorpha senegalensis* were harvested from a river bank at Nkpologu, Enugu State, Nigeria located between 6º51'24"N and 7º23'45"E in July 2018. The sample was taken to the Herbarium of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka, where it was identified by a Plant Taxonomist, Mr Felix Nwafor and voucher sample (PCG/UNN/0335) was deposited in same herbarium.

Processing and Extraction of Plant Material: The leaves were washed with tap water, drained and allowed to dry under shade at room temperature (25 ± 2ºC). The dried leaves were ground with mortar and pestle to a coarse powder. Crude extract was obtained by Soxhlet extraction for 8 hours using 95% methanol as the solvent. The obtained extract was dried in a water bath at 35°C. The extract was stored in an amber bottle and refrigerated until further use.[14]

Botanical and Physicochemical Evaluation

The sample was subjected to various botanical studies including macroscopy, microscopy, histochemistry, and physicochemical analyses.

Macroscopic and microscopic analyses

The sample was thoroughly observed for morphological and organoleptic properties. Microscopic studies began with the leaf epidermal analysis. Here, fragments of the fresh leaves were cleared with 3.5% sodium hypochlorite by soaking for 18 hours. The leaf epidermises were extracted with a sharp razor blade. Transverse section of the leaf was obtained with sledge microtome. Safranin (20%) and glycerin (10%) served as stain and mountant respectively. The prepared slides were viewed and studied under Motic 2.0 camera attached to Motic B3 trinocular light microscope (USA). Quantitative measurements were done with the help of the in-built camera software.[15]

Chemomicroscopic and histochemical analyses

A pinch of the sample powder was cleared with chloral hydrate solution and used for the detection of lignin, calcium oxalates, starch, gum and mucilages, lipids, and proteins by staining with appropriate reagents (Table 1). Stained samples were viewed and studied under Motic 2.0 camera attached to Motic B3 trinocular light microscope (USA) and photomicrographs were taken.[15]

Analysis of physical properties

Physical parameters of the powdered drug were determined to evaluate the loss on drying (moisture content), ash values (total ash, acid-insoluble ash and water-soluble ash) and extractive values (petroleum ether, chloroform, ethylacetate, methanol and water) following standard procedures Onyekere et al.[16] briefly described below:

Loss on drying (moisture content): This was determined by heating a known quantity of the sample in the oven at 105°C until constant weight was achieved. The percentage of the difference between the initial and final weights was calculated as the percentage moisture content.[16]

Ash value: Total ash was estimated by igniting a known quantity of the sample in a muffle furnace at high temperature (800°C) until all the carbon was eliminated. Percentage ash value was calculated in respect to the weight of starting material. Acid insoluble and water soluble ash values were estimated by dissolving a known quantity of the ash in dilute HCl and hot water respectively. They were then filtered and the respective variables were calculated in respect to the weight of starting material.[16]

Extractive values: The sample was tested for its extractive values in petroleum ether, chloroform, ethylacetate, methanol and water. In each case, 5 g of the sample was loaded in soxhlet extractor thimble. Each of the solvents (50 ml) was used to achieve exhaustive extraction of the plant material. Water extractive value was achieved by soaking 2 g of the sample in 25 ml of water for 24 hours with continuous stirring, and filtered. The respective extracts were dried in an evaporating dish using hot air oven at 105ºC until constant weight. This was repeated thrice with each of the solvents and extractive values were calculated in percentage of the starting material.[16]

Phytochemical screening

Qualitative phytochemical screening was carried out according to standard procedures of Sofowora,[17] Trease and Evans[18] and Vijisaral and Subramanian[19] using specific reagent methods as follows: Alkaloids (Dragendorff's reagent), flavonoids (alkaline test), reducing sugars (Fehling's test), saponins (Frothing test), glycosides (Legal's Test), carbohydrates (Molisch's test), steroids (Liebermann-Buchard's test), terpenoids (Liebermann-Buchard's test), tannins (Ferric chloride test) and phenolics (ferric chloride test).

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The methanolic extract of the sample was run on a GC-MS analyzer (Agilent Technologies 7890 USA, equipped with MS detector 5975 Agilent Technologies and HP5 MS capillary column of 30.00 m × 0.320 mm inner diameter × 0.25 µm film thickness). The injection, mass transfer line and ion source were set at 250°C. The oven temperature was programmed from 80°C, held for 2 min at a rate of 10°C/min to 240°C and held for 6 min. Helium was used as carrier gas with a constant flow rate of 2 mL/min. The injected volume of test sample was 1 µL with a split-less mode. The volatile components of the test fractions were identified on the basis of GC retention time on fused silica capillary column, by comparison of their mass fragmentation pattern with literature reports and by computer matching with standard spectra (NIST 2014 version 2.1.0).[16]

In vivo anti-iflammatory assays

Source of Animals: Adult male Wister rats weighing between 95 to 110 grams were purchased from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were housed in groups of 5, at temperature of 25 ±1 °C and fed with commercial animal feed and clean water *ad libitum*.[14]

Carrageenan-induced Edema Model: Animals were randomly divided into 5 groups of 5 rats each: group I: Control (1ml of normal saline); group II: Extract (100 mg/kg b.w., group III: Extract (200 mg/kg b.w., group IV: Extract (400 mg/kg b.w., group V:ibuprofen (100 mg/kg b.w.).

Table 1: Result of powder chemomicroscopy of the leaf of *L. senegalensis*

Parameter	Reagent(s)	Result
Calcium oxalates	Iodine solution	Present; Both prism-shaped and raphides
	Conc. Sulphuric acid	
Cellulose	Zinc chloride;	Present
	Conc. Sulphuric acid	
Gum/Mucilage	Ruthenium red	Absent
Lignified tissues	Conc. HCl + Phloroglucinol	Present
Oil	Sudan III reagent	Present
Starch grains	Iodine solution	Present

The animals were pretreated with drug or extract orally 1 hr before the experiment. A 0.05 ml of 1% carrageenan was aseptically injected into the sub-plantar surface of right hind paw of each rat. Paw edema was measured with venier calipers at 0 hour, 1 hour, 2 hour, 4 hour and at the end of 8 hours. Percentage inhibition/protection against edema formation was taken as the measure of acute anti-inflammatory activity as the difference between the mean size of edema (measured using vernier calipers) in treated animals in respect to the mean size of edema in untreated animals.[20]

Cotton Pellet Induced Granuloma Model: This is chronic anti-inflammatory activity model adopted from Al-Hejjaj *et al*.[20] Briefly, previously sterilised and pre-weighed cotton pellets (10 ± 2 mg) were implanted subcutaneously in the groin of each rat. They were fed *ad libitum* and allowed access to clean water while the respective drugs were administered daily for 14 days. On the 15th day, the animals were sacrificed and the granulation tissues embedded cotton balls were brought out, cleaned of the extraneous tissue and dried in a hot air oven to a constant weight. The weight of granuloma weight was estimated from between dry weight cotton pellets before implantation and dry weight of the granulation tissues embedded cotton balls after implantation. Percentage anti-granuloma activity is the percentage of mean weight of granuloma treated animals in respect to the mean weight of granuloma in untreated animals.

Statistical Analysis

Results obtained from the experiments were processed on Microsoft Excel Spreadsheet (Version 2010) and One-way analysis of variance (ANOVA) followed by Duncan's post hoc test at $P < 0.05$ were carried out using statistical package for social sciences (SPSS) (version 16).[14]

Results and Discussion

Field observations and macroscopy revealed the *Lasimorpha senegalensis* is a herbaceous monocot plant of the arum family and naturally grows in swampy situations. It has broad sagitate leaves (28 – 35 cm long and 13 – 22 cm broad) and long petioles (36 – 42 cm) covered with prickles. The leaves are deep green when fresh but, becomes lighter coloured after drying (Figure 1). Foliar epidermal studies showed that the leaf is amphistomatic (bearing stomata on both the adaxial and abaxial surfaces of the leaves) with more stomata on the abaxial (lower) surface. Stomata were paracytic (had two subsidiary cells lying parallel to the guard cells) with elongated and kidney-shaped guard cells. Epidermal cells are polygonal in shape with straight anticlinal cell wall, which are thicker on the abaxial surface than on the adaxial surface (Figure 2). The transverse section showed the leaf is covered by a cuticle and single-layered epidermis. This is followed by series of column shaped palisade mesophyll cells and spongy mesophylls. The circular vascular bundle (consisting of the phloem, cambium and xylem) and central pith are seen (Plate 1).

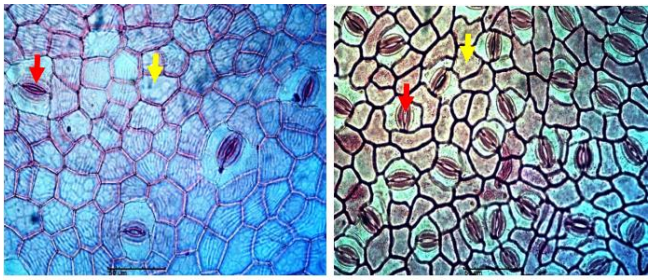
Quantitative stomata parameters on the adaxial and abaxial surfaces respectively were: stomata density (25.00 ± 2.58 mm^{-2} and 175.00 ± 6.49 mm^{-2}), stomata length (29.78 ± 1.51 µm and 26.20 ± 0.75 µm), stomata width (17.74 ± 1.55 µm and 15.47 ± 0.80 µm), stomata size/area (530.21 ± 69.08 $µm^2$ and 405.23 ± 21.11 $µm^2$) and stomata index (3.48 ± 0.34% and 16.83 ± 0.52%). These findings corroborate Vaidya[21] on the stomatal complexes of some members of Araceae and reported parasitic stomata and kidney-shaped guard cells in *Caladium* and *Schismatoglottis* species. Stomata indices are rarely affected by environmental conditions and are therefore relevant in identification and authentication herbal drugs, even when in powdered form.[15, 22] The chemomicroscopy analysis indicated the presence of lignin, calcium oxalate crystals, starch, cellulose, oil and lignin, while gum and mucilage were absent (Table 1). These are also important identification markers.[22] Raphides and calcium oxalate crystals which were observed here are characteristic of the members of this family and are considered the cause of their toxic and irritating properties. However, when properly processed by boiling, the toxic effects of calcium oxalates are reduced to minimal.[12] Therefore, decoction method adopted by the local people is appropriate for preparation this drug is in order to avoid calcium oxalate poisoning in the patients.

The crude drug had appreciable extractive yield with methanol (16.22 ± 0.17%) and ethanol (14.42 ± 0.22) while non-polar pet. ether yielded poorly at 4.23 ± 0.15%. Loss on drying and total ash value were 8.63 ± 0.09% and 6.77 ± 0.09% respectively. These and more physicochemical parameter are presented in Table 2. High total ash value is often an indication of impurities in form of extraneous matter or soil particles in the drug sample. The percentage water soluble ash gives an idea of water exhaustible drugs while the percentage acid insoluble ash indicates the amount of silica present, especially as sand and siliceous earth. High moisture content of a drug sample results to a higher risk of deterioration of the drug sample by microorganisms, which is very important in estimating its shelf-life. Extractive values determine the nature of active constituents present in a given medicinal plant material. The results implied that the drug had more of polar (hydrophilic) compounds than non-polar (hydrophobic) compounds.[16] All these indices are necessary and will be helpful in developing the quality assurance specifications and monograph for this drug.[7]

Preliminary phytochemical screening showed presence of all the tested groups in varying amounts (Table 3). These phytoconstituents are known for different therapeutic functions.

(a) (b)

Figure 1: Macroscopic features of the leaves of *L. senegalensis*: (a) and (b) are habit and sliced/dried leaves photographs respectively



(a) (b)

Figure 2: Leaf epidermal strips of *L. senegalensis*: (a) adaxial surface; (b) abaxial surface; epidermal cells (yellow arrows) and stomata (red arrows) were present on both surfaces

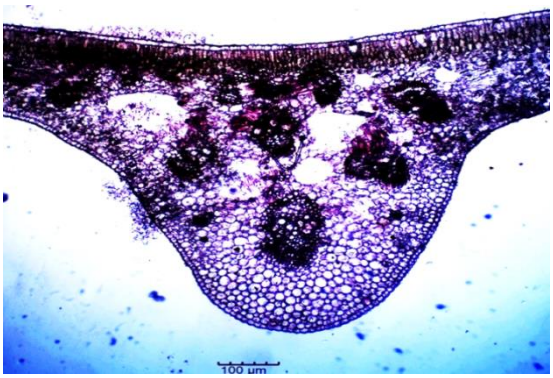


Plate 1: Transverse section of the leaf of *L. senegalensis*

For example, alkaloids exhibit analgesic, antimalarial, antispasmodic, psychostimulant and antimalarial effects; Glycosides and steroids are hormonal and have some effects on fertility; terpenoids tannins, phenolics and flavonoids are polyphenolic compounds known to possess antioxidant, anticarcinogenic, anti-inflammatory and antimicrobial properties; saponins are haemolytic and lower cholesterol.[22] Gas chromatography-mass spectrometry (GC-MS) analysis identified a total of 18 bioactive compounds in the methanolic leaf extract such as terpen-4-ol, phytol, palmitoleic acid, squalene, 4-methyldecosane among others (Table 4). Their chemical structures are also presented in Figure 1. Among these compounds, terpene-4-ol,[2, 23] palmitoleic acid,[24] n-hexadecanoic acid,[1] octadecanoic acid[3] and 22-stigmasten-3-one[25] are proven anti-inflammatory agents. They have also been established to possess other activities such as anticancer,[26] antibacterial,[27] hepatoprotective[28] among others (Table 7).

Inflammation is the integral part of the body's defense mechanism. Acute inflammation is characterized by vasodilatation, exudation of plasma, release of various inflammatory mediators, cytokines, growth factors and emigration of leukocytes.[29] Carrageenan is regarded as an established phlogistic agent/oedemogen and edema induced by the subplantar injection of carrageenan in the rat hind paw is reported to have been inhibited by a number of steroidal and non-steroidal anti-inflammatory drugs.[30] In our study the methanol extract of *L. senegalensis* (100 mg/kg, 200 mg/kg and 400 mg/kg) significantly reduced edema induced by the carrageenan (Table 5). The percent inhibition of paw edema by ibuprofen at the 8th hour was 55.11% while those of LMCE were 24.54%, 52.1% and 54.66% respectively (Figure 2). Hence the extracts (especially at 400 mg/kg) showed anti-inflammatory activity comparable to that of ibuprofen. .

Cotton wool granuloma is a method for testing the proliferative phase i.e granuloma formation, provoked by the subcutaneous implantation of compressed cotton pellets. The percent inhibition of granuloma formation by ibuprofen was 91.79% and that of the extract were 45.24%, 63.32%, 87.59% and 91.79% respectively (Table 6 and Figure 3). Hence the extract showed ($P > 0.05$) anti-granuloma activity as that of ibuprofen ($P < 0.05$). . The additional finding was the body weight loss observed in the ibuprofen group than the LMCE treated animals, suggest that ibuprofen induced gastritis might be responsible for the reduced food intake and leading to loss of weight.[31] Though LMCE showed less chronic anti-inflammatory activity compared to ibuprofen further assessment of LMCE with change in the dosage, solvent extracts and other chronic inflammatory models, will throw more light on its chronic anti-inflammatory activity. These results are in agreement with Jose and Antony,[31] Sabu and Kuttan,[32] Benni *et al*.[29] and Meshram *et al*.[30]

Table 2: Physical parameters of the crude drug (leaf powder of *L. senegalensis*)

Parameter	Mean value (%)
Percentage yield	16.22 ± 0.17
Loss on drying	8.63 ± 0.09
Ash value	6.77 ± 0.09
Acid insoluble ash	2.49 ± 0.02
Water soluble ash	4.00 ± 0.14
Water extractive value	9.00 ± 0.20
Alcohol extractive value	14.42 ± 0.22
Ethyl acetate extractive value	4.23 ± 0.19
Chloroform extractive value	9.03 ± 0.09
Pet. ether extractive value	4.23 ± 0.15

Values expressed as mean ± SEM; n = 3

Conclusion

In conclusion, methanol leaf extracts of *L. senegalensis*, at the tested doses possesses anti-inflammatory activity. and therefore justifies its local use by the indigenous people in management and treatment of inflammatory conditions. The phytochemicals present in the plant could be responsible for these activities. Further studies using different models are recommended to understand the mechanisms of action and explore other techniques such as HPLC etc. in identifying other phytoconstituents.

Conflict of Interest

The authors declare no conflict of interest.

Table 3: Result of preliminary phytochemical screening

Phytoconstituent	Observation
Tannins	+
Flavonoids	+
Alkaloids	+
Hydrogen cyanide	+
Glycosides	+
Phenolics	+
Terpenoids	+
Steroids	+
Saponins	+

+ present

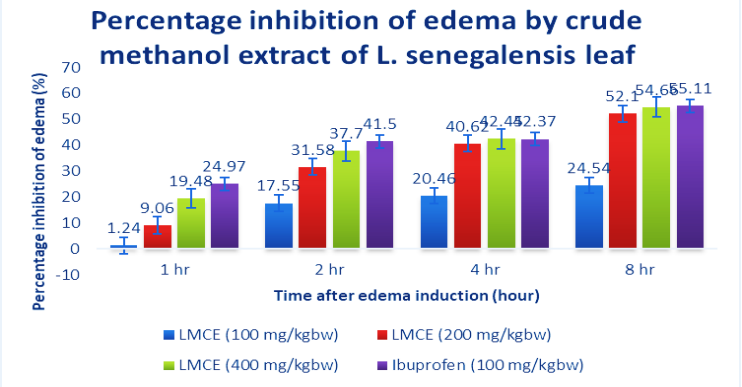


Figure 3: Percentage inhibition of edema by the methanolic extract of *L. senegalensis*

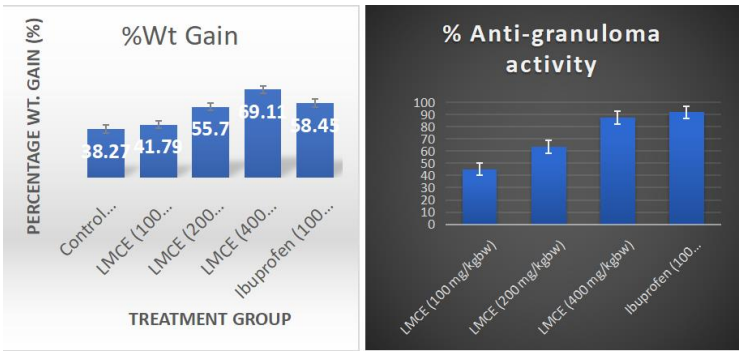


Figure 4: Percentage weight gain and anti-granuloma activities of the methanolic extract of *L. senegalensis*

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 4: GC-MS analysis result of the methanolic leaf extract of *L. senegalensis*

Peak	R. time (min.)	Peak Area (%)	IUPAC Name	Molecular formula	Molecular weight (g/mol)
1	6.62	1.94	Terpinen-4-ol	$C_{10}H_{18}O$	154.25
2	13.87	4.57	6,10,14-Trimethylpentadecan-2-one	$C_{18}H_{36}O$	268.5
3	13.94	2.66	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	$C_{10}H_{18}$	138.25
4	14.15	0.93	3-Azabicyclo[3.2.2]nonane	$C_8H_{15}N$	125.21
5	14.31	1.22	Phytol, acetate	$C_{22}H_{42}O_2$	338.6
6	14.66	1.62	Palmitoleic acid	$C_{16}H_{30}O_2$	254.41
7	14.87	16.10	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241
8	16.16	20.36	Phytol	$C_{20}H_{40}O$	296.5
9	16.23	8.89	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4455
10	16.30	17.25	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614
11	16.50	5.63	Octadecenoic acid	$C_{18}H_{34}O_2$	282.5
12	17.87	1.00	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	234.133
13	18.96	1.23	3-Eicosene, (E)-	$C_{20}H_{40}$	280.5316
14	19.98	3.17	22-Stigmasten-3-one	$C_{29}H_{48}O$	412.7
15	20.21	5.51	1,2,15-Pentadecanetriol	$C_{15}H_{32}O_3$	260.41
16	20.32	3.13	Squalene	$C_{30}H_{50}$	410.730
17	20.44	4.14	8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene	$C_{15}H_{24}$	204.35
18	20.67	0.65	4-Methyldocosane	$C_{23}H_{48}$	324.6

Table 5: Mean paw diameter of the animals at different periods

Treatment group	0 Hour	1 hr	2 hr	4 hr	8 hr
Control (Untreated)	0.55 ± 0.03	0.60 ± 0.04	0.63 ± 0.02	0.62 ± 0.02	0.59 ± 0.01
LMCE (100 mg/kgbw)	0.56 ± 0.01^{a}	0.61 ± 0.03^{a}	0.51 ± 0.01^{b}	0.49 ± 0.02^{c}	0.44 ± 0.02^{c}
LMCE (200 mg/kgbw)	0.53 ± 0.01^{a}	0.55 ± 0.02^{b}	0.43 ± 0.02^{c}	0.37 ± 0.02^{cd}	0.28 ± 0.01^{d}
LMCE (400 mg/kgbw)	0.51 ± 0.01^{a}	0.48 ± 0.02^{b}	0.39 ± 0.02^{c}	0.36 ± 0.01^{c}	0.27 ± 0.01^{c}
Ibuprofen (100 mg/kgbw)	0.55 ± 0.01^{a}	0.45 ± 0.02^{b}	0.36 ± 0.01^{c}	0.36 ± 0.01^{c}	0.26 ± 0.01^{c}

Values expressed as mean ± SE; n = 5
Means with different letters as superscripts are significantly different at $p \leq 0.05$
LMCE = Lasimorpha methanol crude extract

Table 6: Mean animal weight and granuloma weight among the treatment groups

Treatment group	Initial weight (g)	Final weight (g)	Weight gain (g)	Wt. of granuloma (mg)
Control (Untreated)	104.74 ± 3.23	133.04 ± 4.88	28.30 ± 4.23	78.60 ± 5.10^{a}
LMCE (100 mg/kgbw)	101.84 ± 4.23^{a}	131.94 ± 7.01^{b}	30.10 ± 3.54^{d}	42.00 ± 2.81^{b}
LMCE (200 mg/kgbw)	109.48 ± 3.53^{a}	153.28 ± 3.94^{a}	43.80 ± 2.39^{b}	28.00 ± 1.87^{c}
LMCE (400 mg/kgbw)	98.12 ± 5.98^{a}	143.22 ± 6.90^{a}	45.10 ± 3.29^{a}	10.00 ± 5.48^{d}
Ibuprofen (100 mg/kgbw)	93.64 ± 3.98^{b}	130.68 ± 6.43^{b}	37.04 ± 3.78^{c}	6.00 ± 0.24^{e}

Values expressed as mean ± SE; n = 5
Means with different letters as superscripts are significantly different at $p \leq 0.05$
LMCE = Lasimorpha methanol crude extract

Table 7: Reported pharmacological activities of the compounds revealed by GC-MS analysis of the leaf of *L. senegalensis*

Peak	Compound	Pharmacological Activities
1	Terpinen-4-ol	Anti-inflammatory,[2, 23] anticancer,[26] antibacterial,[27, 33, 34] antimicrobial[35]
2	2-Pentadecanone, 6,10,14-trimethyl	No reported activity
3	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	Antimicrobial,[36] antioxidant[37]
4	3-Azabicyclo[3.2.2]nonane	Anti-protozoal,[38, 39] antiplasmodial, antitrypanosomal[40]
5	Phytol, acetate	CNS stimulant[41]
6	Palmitoleic acid	Prevents beta cell apoptosis, lowers LDL cholesterol,[42] anti-inflammatory,[24] antidiabetic[43]
7	n-Hexadecanoic acid	Anti-inflammatory,[1] anticancer,[44] antioxidant, hypocholesterolemic, nematicide, anti-androgenic, flavor, hemolytic[28]
8	Phytol	Antinociceptive, antioxidant,[45] antimicrobial[46]
9	9,12-Octadecadienoic acid (Z,Z)-	Hypocholesterolemic, anti-eczemic, hepatoprotective, antihistaminic[28]
10	9-Octadecenoic acid	Hypocholesterolemic, anti-eczemic, hepatoprotective, antihistaminic[28]
11	Octadecenoic acid	Antimicrobia[47] anti-inflammatory[3]
12	2-Piperidinone, N-[4-bromo-n-butyl]-	Antimicrobial,[48] anti-inflammatory[49]
13	3-Eicosene, (E)-	Antioxidant,[50] antimicrobial,[51] antihyperglycemic, cytotoxic,[52] insecticidal[53]
14	22-Stigmasten-3-one	Antimicrobial, anticancer, anti-inflammatory,[25] antiarthritic, antiasthama, diuretic[54]
15	1,2,15-Pentadecanetriol	No reported activity
16	Squalene	Antibacterial, antioxidant, antitumor, cancer preventive,[55] immunostimulant, chemo-preventive, lipoxygenase-inhibitor, pesticide[53, 56, 57, 58]
17	8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene	No reported activity
18	4-Methyldocosane	No reported activity

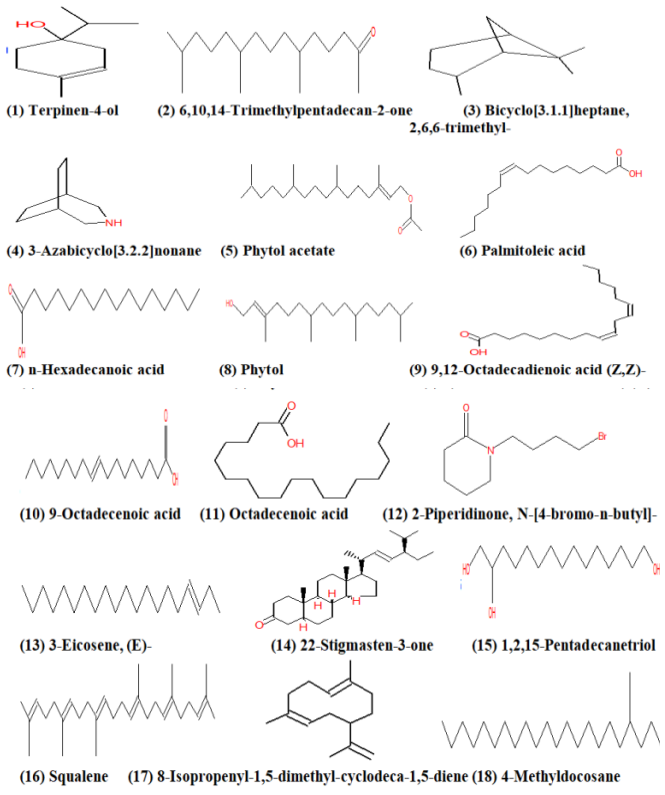

Figure 5: Molecular structures of the compounds identified through GC-MS analysis

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