Studies on Phytochemicals and Bioactivities of methanolic extract of *Leucaenaleucocephala*(Lam.) de Wit Fruit Pods

ABSTRACT

Objectives: The study was designed to screen for the phytochemical constituents, and investigate the antioxidant, antibacterial, and anti-inflammatory activities of *Leucaenaleucocephala* fruit pods methanolic extract (LLFPME).

Materials and Methods: Matured and dried fruit pods were pulverized and extracted with methanol using maceration method. The filtrate was concentrated in a rotary evaporator under reduced pressure to obtain powdery form termed LLFPME. The LLFPME was tested for the presence of phytochemicals using TLC and GC-MS technique. The phenolic and flavonoid contents of the extract were quantified; the antioxidant potential of the extract was examined using DPPH and H_2O_2 radical scavenging, metal chelating, FRAP and TAC assays. The anti-inflammatory potential was evaluated using the membrane stabilizing and lipoxygenase inhibitory activities while the antibacterial activity was investigated bydetermining the zone of inhibition, minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of extract against the test bacterial isolates.

Results: TLC revealed the presence of flavonoids, saponins, alkaloids, tannins, and others while the GC-MS analysis also showed the presence of 27 compounds. It was shown that the LLFPME displayed measurable antioxidant activity by exhibiting DPPH and H_2O_2 free radical scavenging potential and dose dependent metal chelating ability. FRAP and TAC were estimated to be 14.88 ± 0.04 and 25.53 ± 0.21 mgAAE/g of extract, respectively. The extract protected the RBC membrane integrity of RBC subjected to heat and hypotonic stress; and inhibited the activity of lipoxygenase.The extract inhibited the growth of some bacterial strains but had no visible effect on *Pseudomonas aeuroginosa*growth. The zone of inhibition, MIC and MBC exhibited by the extract against susceptible test bacteria ranges between 13 - 17 mm, 2.5 - 20 and 20 mg/ml, respectively..

Conclusion: It was concluded that LLFPME exhibited antioxidant potential through various mechanisms that possibly aided its ability to stabilize stressed cell membrane and similarly inhibited the growth of some bacterial strain.

Key words: *Antioxidants, anti-inflammatory, anti-bacterial, gas chromatography-mass spectrometry, thin-layer chromatography*

1. INTRODUCTION

Medicinal plants have gained significant attention over the years due to the various bioactive compounds present in them, whichhave been proven to promote health benefits in the treatment and management of ailments and diseases [1].“These bioactive compounds have been found and reported to show a wide range of biological activities including antimicrobial, antioxidant, anticancer, anti-inflammatory, anti-hyperlipidemiaamong others” [1,2]. Owning to their wide range of use, antioxidants have been explored in the treatment and management of many ailments such as cardiovascular as well as inflammatory diseases[3,4].

Due to economic downturn in Nigeria and developing countries over a period of time, there has been increased shift of attention to the use of medicinal plants which are adjudged to be relatively safe for human health [1,3,5].

Leucaenaleucocephala, a perennial legume belonging to the fabales order and fabaceae family of plant, is widely used as a shield for cash crops like cocoa and coffee. It has an intermediate sized leaflets and large pods in crowded clusters. The leaf of the plant has been reported to possess medicinal properties which range from antidiabetic, antihelmintic, anticancer, and control of stomach diseases [6]. These properties have been attributed to its phytoconstituents such as phytol, squalene, [7] and bioactive active compounds like flavonoids, tannin and alkaloids [8]. Various parts of the plant such as the root, leaves, stem-wood, stem-bark and fruits have shown biological activities[9-11]. However, the antioxidant, anti-inflammatory and antibacterial properties of the fruit pods have not been reported, hence the present study was designed to investigate these properties.

2. MATERIALS AND METHODS

2.1 Chemical, reagents and media

Quercetin, 1,1-diphenyl-2-picrylhydrazine, gallic acid, Folin-Ciocalteu reagent were purchased from Sigma Chemical (Sigma-Aldrich Co., St. Louis, MO, USA). All other chemicals and media used were of analytical grade.

2.2 Collection of *L. leucocephala* fruit pods

*Leucaenaleucocephala*fruit pods were collected from the Botanical Garden, Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria. Identification of the fruits was done in Ife Herbarium, Botany Department of the university. The voucher number is 18260.

2.3 Preparation of *L. leucocephala*fruit pods methanolic extract.

*Leucaenaleucocephala*fruit pods were separated from the seed; they were washed with distilled water, air-dried and powdered using warring blender. 300 g of the powdered fruit pods of *L. leucocephala* were soaked in 3 L of 80% methanol for 72 hours. The mixture was filtered using cheese cloth and later Whatman filter paper No 1. The filtrate was concentrated using a rotary evaporator at 40ºCunder reduced pressure. The concentrate was later freeze dried to obtain the powdery form. The powdery form was termed *L. leucocephala*fruit pod methanolic extract (LLFPME) and used for biological assays.

2.4 Phytochemical Screening

Qualitative phytochemical analysis was carried out on the extract using Thin Layer Chromatography and Gas Chromatography-Mass Spectrometry to detect and identify the presence of phytochemicals.

2.5 Thin layer chromatography (TLC) of LLFPME

Thin layer chromatography profile of the LLFPME was carried out using silica gel precoated plates by one-way ascending technique. The TLC plates were activated in hot air oven at 120ºC for 3 min. The LLFPMEwas applied to the origins of a TLC plate 0.5 cm above its bottom using capillary tubes. After the application of the extract on the plate, the plates were kept in a small-saturated chromatographic tank containing the solvent system. Mobile phase was allowed to move through adsorbent phase up to three-quarter of the plate. Each chemical constituent moves up at different rates depending on its solubility in the mobile phase and the strength of its absorption to the stationary phase.

2.6 Gas Chromatography- Mass Spectrometry (GC-MS) Analysis

GC-MS analysis of the extract was performed using an Agilent 5977B GC/MSD system coupled with Agilent 8860 auto-sampler, a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-5MS (5% diphenyl/95% dimethyl polysiloxane) fused to a capillary column (30 × 0.25 µm ID × 0.25 µmdf). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1µl was employed (a split ratio of 10:1). Five (5) point serial dilution calibration standards (1.25, 2.5, 5.0, 10.0 ppm) were prepared from the stock solution of 40 ppm and used to calibrate the GC-MS.

The injector temperature was maintained at 300°C, and the ion-source temperature was 250°C, and the oven temperature was programmed from 100°C (isothermal for 0.5 min), with an increase of 20°C/min to 280°C (2.5 min), Mass spectra were taken at 70 eV; a scanning interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 3 min, and the total GC/MS running time was 21.33 min.

2.7 Total phenolic content (TPC) determination

The total phenol content was determined with the FolinCiocalteu's assay using gallic acid as standard. In the procedure, 0.5 ml of LLFPME was mixed with 1.5 ml Folin-Ciocalteu's reagent (FCR) diluted 1:10 v/v then after 5 min, 1.5 ml of 7% sodium carbonate solution was added. The final volume of the tubes was made up to 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. The experiment was repeated three times for precision and values were expressed in terms of phenol content (Gallic acid equivalent, GAE) per g of dry weight.

2.8 Total flavonoid content (TFC) determination

Total flavonoid content was determined by Aluminium chloride method using quercetin as a standard. LLFPME(1 ml) and 4 ml of water were added into a beaker. 0.3 ml of 5% Sodium nitrite and 0.3 ml of 10% Aluminum chloride was added after 5 min. After 6 min incubation at room temperature, 1 ml of 1 M Sodium hydroxide was dispensed to the reaction mixture and the final volume was made up to 10 ml with distilled water. Absorbance was measured against the blank at 510 nm using a spectrophotometer. The

experiment was repeated three times for precision and values were expressed in terms flavonoid content (Quercetin equivalent, QAE) per g of dry weight.

2.9 Assessment of antioxidant potential of LLFPME

2.9.1 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging potential of LLFPMEwas evaluated using the amended method [12]. 1 ml of DPPH working solution (0.01 mM DPPH in 95% methanol) prepared few minutes before the commencement of the experiment was added to 1 ml of various concentrations (0.0 – 1 mg/ml final concentration) of LLFPME. The test-tubes were placed in the dark for 30 min at 25ºC and thereafter, reading was taken at a wavelength of 517 nm in Biobase UV-visible spectrophotometer BK-D5 series. The blank has distilled water in place of the extract and ascorbic acid was used as standard antioxidant agent. The equation below was used to calculate the DPPH radical scavenging ability of LLFPME in percentage.

$$\text{DPPH radical Scavenging (\%)} = \frac{Absorbance_{\ blank} - Absorbance_{\ sample}}{Absorbance_{\ blank}} \text{ x } 100$$

2.9.2 Hydrogen peroxide (H_2O_2) radical scavenging activity assay

The hydrogen peroxide radical scavenging capability of LLFPMEwas carried out based on the revised procedure [13]. One milliliter of various concentrations (0.0 – 100 µg/ml final concentration) of LLFPME was added to 1.5 ml of 40 mM H_2O_2 solution and vortexed. The reaction proceeded for 15 min after which absorbance was taken at a 230 nm wavelength in UV/Visible spectrophotometer. The blank solution contains distilled water as a replacement for the sample. Aspirin was employed as positive control.

$$\text{Hydrogen peroxide Scavenging (\%)} = \frac{Absorbance_{\ blank} - Absorbance_{\ sample}}{Absorbance_{\ blank}} \text{x } 100$$

2.9.3 Metal chelating activity assay

The ferrous ion chelation of LLFPMEwas evaluated based on iron-ferrozine reaction. The procedure [14]was adopted for this assay with slight adjustment. The LLFPME (0.0 – 1.0 mg/ml) at varying final concentrations was mixed with 0.5 ml of 2 mM $FeSO_4.7H_2O$ and 2 ml of 0.25 mMferrozine. The mixture was gently shaken and kept for 10 minutes at room temperature. The absorbance was taken at 562 nm wavelength. Distilled water replaced the sample in the blank. Positive control assay contained EDTA as

standard chelating agent. The percentage of metal chelating ability was calculated from the equation below:

Metal chelating activity (%) = $\frac{Absorbance_{\ blank} - Absorbance_{\ sample}}{Absorbance_{\ blank}}$ x 100

2.9.4 Ferric reducing antioxidant power (FRAP) assay

The estimation of FRAP ability of LLFPME was based on the procedure [15] with slight modification. Acetate buffer (300 mM, pH 3.6), 2, 4, 6-tri-(2-pyridyl)-1, 3, 5-triazine (TPTZ, 10 mM) dissolved in 40 mMHCl and $FeCl_3.6H_2O$ (20 mM) were mixed in the ratio of 10:1:1 respectively. This mixture was used as the working FRAP reagent. Known volumes of LLFPME (1.0 mg/ml) were pipetted and mixed with FRAP reagent prepared just before use. The mixture was agitated and allowed to stand for 10 min, then absorbance was taken at 593 nm wavelength and compare to that of the reagent blank that contains no sample but distilled water. Absorbance was taken in the dark at ambient temperature. Reducing antioxidant power was expressed as equivalent concentration (EC) which is defined as the concentration of antioxidant that gave a ferric reducing ability equivalent to that of the ascorbic acid standard.

2.9.5 Total antioxidant capacity (TAC) assay

The total antioxidant capacity assay procedure [16] was adopted to evaluate the ability of LLFPME to convert molybdate (IV) to molybdate (V). Briefly, 3 ml of the freshly prepared working solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate.) was added to 0.5 ml of the LLFPME (1 mg/ml) in test-tube. The tube was kept in a regulated water bath set at 95ºC for 90 min. The mixture was chilled to room temperature under running tap water. The absorbance was read at 695 nm wavelength in UV/Visible spectrophotometer against a blank which contain distilled water instead of the plant extract. The antioxidant activity of LLFPMEwas also expressed as an ascorbic acid equivalent.

2.10 Assessment of anti-inflammatory potential of LLFPME

2.10.1 Membrane Stabilizing Activity

The membrane stabilizing activity assay was carried out as previously described [17] using 0.25% (v/v) hyposaline and 0.9% normal saline suspension. The assay mixtures consisted of 1.0 ml of hyposaline (0.25% v/v), 1.0 ml of 0.15 M sodium phosphate buffer, pH 7.4, 0.5 ml of 0.9% (v/v) normal saline, 0.0 - 1.0 ml of drugs (standard, LLFPME) and final reaction mixtures were made up to 3.0 ml with hyposaline.

Drugs were omitted in the blood control, while the drug control did not contain the erythrocyte suspension. The reaction mixtures were incubated at 56^{o}C for 30 min on a water bath, followed by centrifugation at 3000 rpm for 10 min at room temperature. The absorbance of the released hemoglobin was read at 560 nm.

2.10.2 Lipoxygenase Inhibitory Activity

Lipoxygenase inhibitory activity of LLFPME with linoleic acid as a substrate was measured with a UV-VIS spectrophotometer as reported[18] with some modifications. LLFPME was screened for lipoxygenase inhibitory activity at various concentrations (0 - 0.5 mg/ml). The assay mixture consisted of 150 µl phosphate buffer (0.067 M, pH 7.5), 50 µl of each LLFPME solution and 50 µl enzyme solutions (0.28 U/ml in the phosphate buffer). The reaction was initiated by adding 250 µl of substrate solution (0.15 mM in water). Enzymatic kinetic was recorded at 234 nm for 2 min. Negative control was prepared and contained 1% (v/v) methanol solution without LLFPME solution. Quercetin, which was known to inhibit lipoxygenase was used as a standard (positive control) at various concentrations (0 - 0.1 mg/ml). All experiments were performed in triplicate. Lipoxygenases inhibitory activity was calculated using the expression below:

$$(\%)\ \text{inhibition} = \left(\frac{1-B}{A}\right)\times 100$$

Where A is the change in absorbance of the assay without the extracts (negative control) and B is the change in absorbance of the assay with LLFPME. Lipoxygenase inhibitory activity was expressed as the percentage inhibition of lipoxygenase.

2.11 Antibacterial Assays

2.11.1 Zone of inhibition Determination

Agar-well diffusion method [19]was used to determine the antibacterial activity of LLFPME. The bacterial strains were revivedby sub-culturing them in sterile nutrient broth test tubes which were incubated for a period of 18 hours at 37 ºC. The bacterial culture turbidity was later adjusted to 0.08 - 0.1 optical density at 625nm (0.5 McFarland) prior use. The standardized organisms were seeded onto sterile Mueller-Hinton agar plates. Wells were bored into the agar medium using a sterile cork-borer and the resulting wells were carefully filled up with 0.12 ml at concentration 20 mg/ml of LLSPM. Spillage of the LLFPME

solution onto the agar surface was avoided. The plates were left for 60 min on the bench for adequate diffusion of the sample solution through the matrix of the agar before incubating them for 24 hours at 37ºC. The plates were observed for zones of inhibitionafterwards.

2.11.2 Minimum inhibitory concentration

The LLFPME sample minimum inhibition concentration was determined by applying the procedure [19]. Two milliliters of different concentrations of the solution obtained after two-fold dilution of LLFPMEwas mixed with 18 ml of already sterilized moltennutrient agar resulting in a mixture with concentration band of 0.156 mg/ml - 20 mg/ml. Thereafter, the mixture was emptied into sterile petri dishes and allowed to set. The plates wereleft overnightto ensure a dry surface before streaking onto the plates 18 hours oldtest bacteria strains whose turbidity has been adjusted to 0.5 McFarland. The petri dishes were then placed in the incubator for 72 hours at 37ºC. Thereafter, the lines of streak on each plate were observed for growth or absence of growth. The least concentration that gave no room for growth of the bacterial strain was regarded as the minimum inhibitory concentration.

2.11.3 Minimum bactericidal concentration (MBC)

The MBC of the LLFPMEwas evaluated using the method [20]. The innocula for this assay were gotten from the lines of streakon MIC plates specifically the ones without observable growth and thenaseptictransferred by streaking onto newly prepared nutrient agar plates devoid of LLFPMEor any antimicrobial agent. This was followed by 48 hours period of incubation at a temperature of 37ºC. The MBC was taken as the lowest concentration of LLFPME that did not allow any bacterial growth on the surface of the agar plates at the end of the 48 hours incubation period.

2.12 Statistical Analysis

The data were analyzed using GraphPad prism 5.0 and expressed as the mean ± standard error of mean (SEM) of triplicate experiments.

3. RESULTS

3.1 Percentage yield of LLFPME

The percentage yield of LLFPME obtained was 5.16 g representing a 1.58% of the starting material.

3.2 Phytochemical constituents of LLFPME

3.2.1 Thin Layer Chromatography

Table 1 shows the secondary metabolites present in LLFPME. The secondary metabolites present using the TLC are alkaloids, flavonoids, tannins, triterpenes, cardiac glycoside, saponins and steroids with flavonoids the most abundant.

3.2.2 GC-MS Analysis

Chromatogram of the GC-MS analysis of LLFPME is shown in Figure 1. The profile shown in Table 2 revealed 27 compounds in LLFPME, with reported biological activites such as antioxidant, anti-microbial and anti-inflammatory. The most abundant are 9-Octadecenoic acid, Z-methyl ester (16.78%), 14-methyl-pentadecanoic acid (8.98%) and Docosanoic acid (8.05%).

Table 1: The phytochemicals revealed by TLC

Phytochemicals	Present/Absent
Alkaloids	+
Flavonoids	+
Tannins	+
Triterpenes	+
Cardiac glycosides	+
Saponins	+
Steroids	+

+ stands for present while – stands for absent

Figure 1: Gas chromatogram of *L. leucocephala* fruit pod methanolic extract

Table 2: GCMS profile of *L. leucocephala* fruit pod methanolic extract

S/N	Retention	Area %	Compound name
1	3.213	2.51	Methyl pyrrole 2-Carboxylate
2	3.333	2.65	3H-pyrazol-3-one
3	4.529	4.17	Undecane
4	4.638	3.58	Undecane
5	5.376	1.64	Dodecamethyl-cyclohexasiloxane
6	6.240	1.53	Dodecamethyl-cyclohexasiloxane
7	6.463	1.84	Dodecamethyl-cyclohexasiloxane
8	7.316	2.33	2-methoxyl-4-vinylphenol
9	8.781	1.64	Tetradecamethyl-cycloheptasiloxane
10	8.946	2.07	Tetradecamethyl-cycloheptasiloxane
11	9.004	2.91	Octasiloxane
12	9.233	2.15	Tetradecamethyl-cycloheptasiloxane
13	10.285	1.39	Diethyl phthalate
14	12.517	2.82	(S)(+)-Z-13-Methyl-11-pentadecen-1-ol acetate
15	12.563	2.18	Pentadecyne
16	12.786	2.87	2-(2’,4’,4’,6’,6’,8’,8’-Heptamethyltetrasiloxan-2’-yloxyl)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane
17	13.187	8.98	14-methyl-pentadecanoic acid
18	14.136	8.05	Docosanoic acid, methyl ester
19	14.543	4.16	9,12-Octadecadienoic acid(Z,Z)-methyl ester
20	14.588	16.78	9-Octadecenoic acid Z-methyl ester
21	14.628	1.84	Oleic acid
22	14.772	5.56	Methyl Stearate
23	15.578	3.02	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-

			hexadecamethyl
24	18.313	1.39	Oleyl alcohol, trifluoroacetate
25	19.012	3.17	Oleic Acid
26	21.077	7.39	Cholesta-6,22.24-triene, 4,4-dimethyl
27	21.140	1.56	Cholesta-6,22.24-triene, 4,4-dimethyl

3.3 TPC and TFC of LLFPME

The summary of the flavonoids and phenolics concentration of LLFPME is presented in Table 3. The findings showed that the fruit pod of the plant is rich in flavonoids and phenolics, important bioactive compounds with antioxidant and anti-inflammatory activities.

Table 3: TPC and TFC of *L. leucocephala* fruit pod methanolic extract

Assay	Value
Total phenolics (mgGAE/g)	1.36 ± 0.13
Total Flavonoids (mgQE/g)	41.68 ± 0.10

Each value represented the Mean ± SEM of 3 readings

3.4 Antioxidant activities of *L. leucocephala* fruit pod methanolic extract

3.4.1 DPPH radical scavenging activity

Figure 2 shows the DPPH radical scavenging activity of LLFPME. The extract of the fruit pod showed potent DPPH scavenging activity (with IC_{50} value of 0.14 ± 0.13 mg/ml)in a concentration dependent manner but lower than that of ascorbic acid (with IC_{50} value of 0.02 ± 0.01 mg/ml), the standard used.

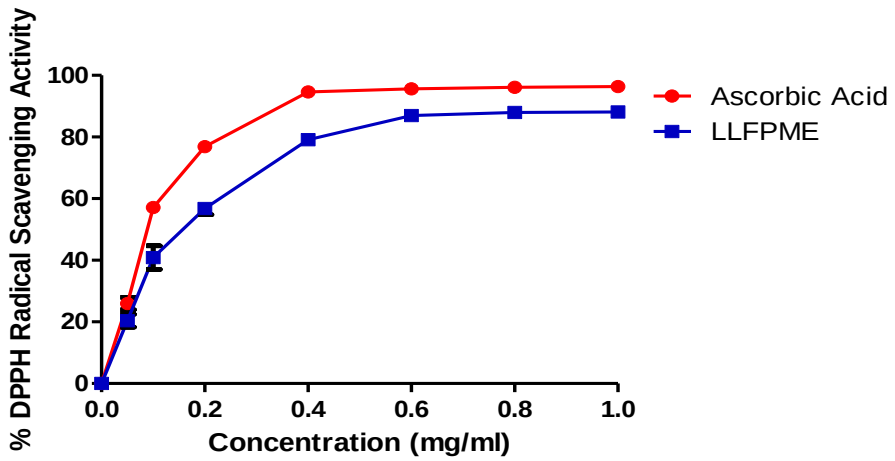

Figure 2: DPPH radical scavenging activity of *L.leucocephala*fruit pods methanolic extract

3.4.2 Hydrogen peroxide scavenging ability

The hydrogen peroxide scavenging assay of LLFPME shows a dose dependent activity with IC_{50} of 182.83 ± 9.79 mg/ml, but not as potent as the standard, aspirin with IC_{50} of 107.68 ± 1.46 mg/ml as shown in Figure 3.

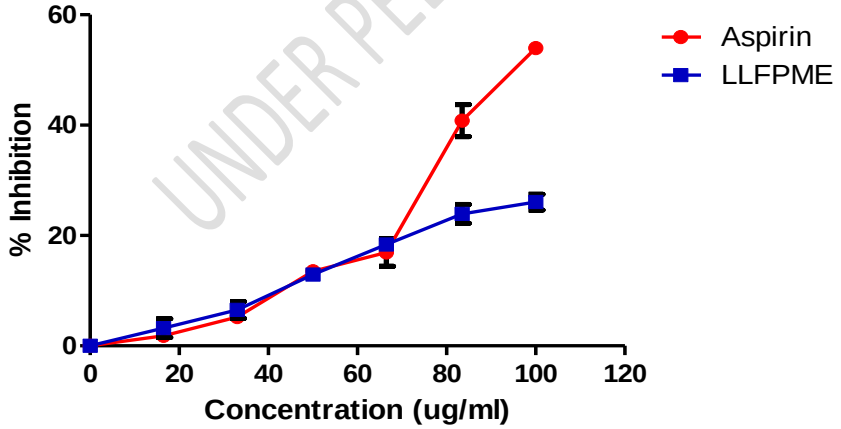

Figure 3: Hydrogen peroxide scavenging activity of *L.leucocephala*fruit pods methanolic extract

3.4.3 Metal (Ferrous ion) chelating ability

The ferrous ion chelating ability of LLFPME as shown in Figure 4 was concentration dependent. The metal chelating ability of LLFPME with IC_{50} value of 0.030 ± 0.002 mg/ml was appreciable and potent; and compared favourably with the standard chelating agent (EDTA) with IC_{50} value (0.046 ± 0.002 mg/ml).

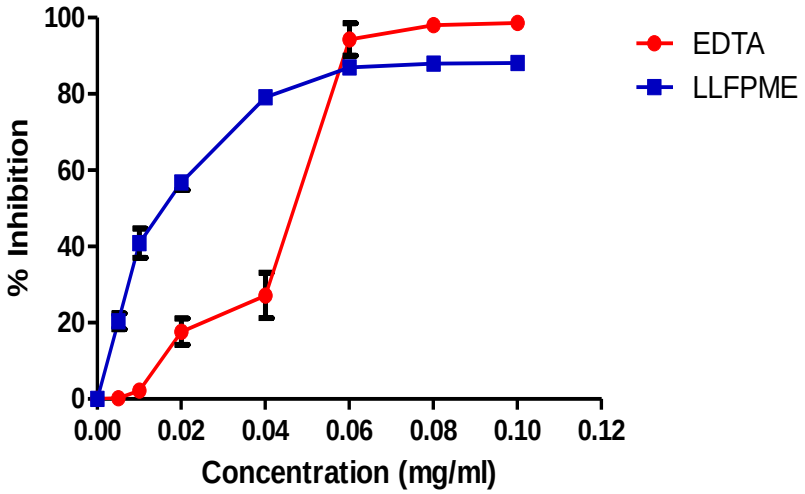

Figure 4: Metal chelating ability of *L. leucocephala*fruit pods methanolic extract

3.4.4 Ferric reducing antioxidant power (FRAP)

The ability of LLFPME to reduce iron (III) to iron (II) was estimated and expressed as mg ascorbic acid equivalent per g of extract (mgAAE/g). The reducing ability of LLFPME was 14.88 ± 0.04 mgAAE/g as shown in Table 4. This shows that the fruit pod of *L. leucocephala* is a source of antioxidant.

3.4.5 Total antioxidant capacity (TAC)

The ability of LLFPME to reduce molybdenum (VI) to molybdenum (V) and subsequent formation of green complex at an acidic pH was expressed as mg equivalent of ascorbic acid per g of extract. The total antioxidant capacity value of 25.53 ± 0.21 mgAAE/g in Table 4 shows that the extract has reducing properties associated with the ability of the compound present to donate hydrogen atom.

Table 4: FRAP and TAC of *L. leucocephala*fruit pod methanolic extract

Assay	Value
FRAP (mgAAE/g)	14.88 ± 0.04
TAC (mgAAE/g)	25.53 ± 0.21

Each value represented the Mean ± SEM of 3 readings

3.5 Anti-inflammatory activities of *L. leucocephala* fruit pod methanolic extract

3.5.1 Membrane Stabilizing Activity

The LLFPME showed 62% maximum and 9% minimum stabilizing ability for the red blood cell exposed to hypotonic and heat stress in a monophasic mode of action (Figure 5).

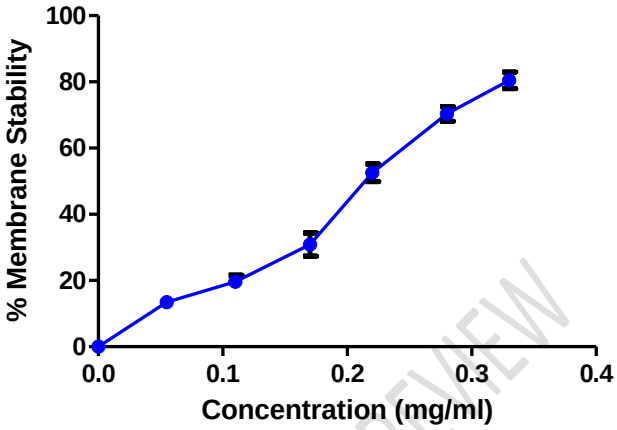

Figure 5: Membrane stabilizing ability of *L. leucocephala*fruit pods methanolic extract

3.5.2 Lipoxygenase Inhibitory Activity

The inhibitory activity of LLFPME against lipoxygenase is shown in Figure 6. The inhibitory activity is dose dependent and appreciable with IC_{50} value of 1.97 ± 0.19 mg/ml but not as potent as that of quercetin (0.04 ± 0.00 mg/ml), the standard agent used.

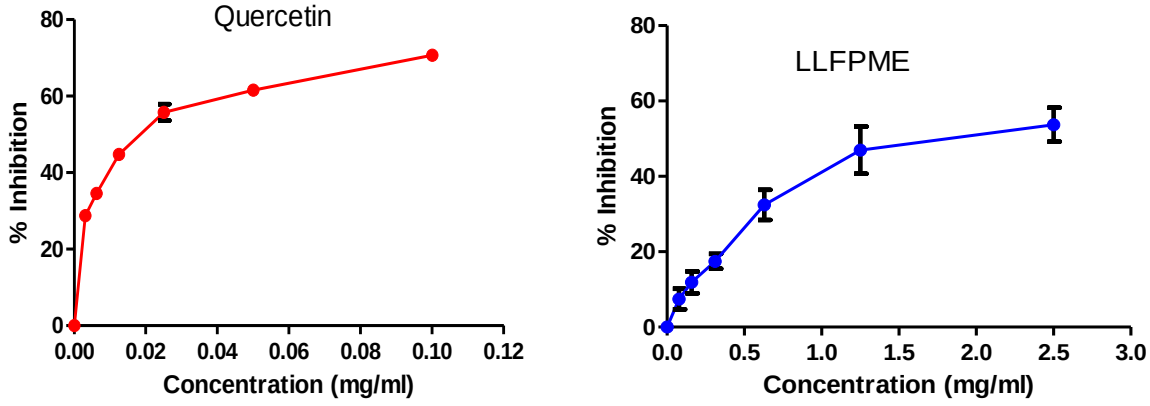

Figure 6: Lipoxygenase inhibitory ability of *L. leucocephala*fruit pods methanolic extract

3.6 Antibacterial Assays

3.6.1 Zone of Inhibition Determination

The sensitivity assay of LLFPME was assessed using Agar disc diffusion method. Table 5 shows the zone of inhibition of the plant fruit pod against *Clostridium sporogens*, *Bacillus cereus,Klebsiella pneumonia*, *Bacillus stearothermophillus* and *Bacillus subtilis* but had no effect on *Pseudomonas aeuroginosa*growthat 20 mg/ml and the LLFPME shows maximum inhibition against *Bacillus cereus* (16.00 ± 0.00 mg/ml).

3.6.2 Minimum Inhibitory Concentration (MIC)

The MIC of LLFPME shown against the bacterial strains ranged between 1.25 mg/ml and 10 mg/ml as presented in Table 5. The least minimum inhibitory concentration value of 1.25 mg/ml was exhibited against *Bacillus cereus* and the peak minimum inhibitory concentration value of 10 mg/ml was against *Clostridium sporogens,Bacillus stearothermophillus* and *Bacillus subtilis.*

3.6.3 Minimum Bactericidal Concentration (MBC)

The MBC shown against the bacterial strains ranged between 5 mg/ml and above 20 mg/ml as presented in Table 5. The least minimum bactericidal concentration value of 5 mg/ml was exhibited against *Bacillus cereus* and the peak minimum bactericidal concentration value of above 20 mg/ml was exhibited against *Bacillus stearothermophillus* and *Bacillus subtilis.*

Table 5: Zone of Inhibition, MIC (mg/ml) and MBC (mg/ml) of *L. leucocephala* fruit pods methanolicextract

		LLFPME			Streptomycin		
S/N	**Test micro-organism**	**Zone of inhibition (mm)**	**MIC (mg/ml)**	**MBC (mg/ml)**	**Zone of inhibition (mm)**	**MIC (µg/ml)**	**MBC (µg/ml)**
1	*Clostridium sporogenes*	15	10	10	16	5	5
2	*Bacillus cereus*	16	1.25	5	13	2.5	2.5
3	*Klebsiella pneumonia*	14.5	2.5	10	NVG	ND	ND

4	*Bacillus stereothermophillus*	13	10	>20	16	2.5	2.5
5	*Bacillus subtilis*	15	10	>20	NVG	ND	ND

Key: NVG = no visible growth, ND = Not Determined.

4. DISCUSSION

Phytochemicals are bioactive compounds synthesized by plants for the protection against invaders but have been employed by humans in the treatment and management of ailments related to oxidative stress, inflammation and pathogens infection [2,3,8]. “Naturally occurring phenolics and flavonoids are ubiquitous in plant parts, possessing diverse properties from antioxidants to anti-inflammatory and anti-microbial. In this study, the phytochemicals of LLFPME was screened using the TLC revealing the presence of flavonoids, saponins, tannins, alkaloids, triterpenes, steroids and cardiac glycosides which have been reported to have diverse biological activities such as antioxidant and anti-inflammatory activities” [21,22]. The GC-MS analysis revealed the presence of twenty-seven phytocompounds, which cut across various chemical classes. The ester chemical class was the highest with regard to an individual presence and 9-Octadecenoic acid (Z)-methyl ester, 14-methyl–pentadecanoic acid andDocosanoic acid-methyl ester were found in highest amount respectively. These compounds have been reported to possess biological activities which include but not limited to cancer prevention, antioxidant, antiandrogenic, antibacterial, antiinflammatory, 5-alphareductase inhibitory and insecticidal [23-27].

The potency of an antioxidant agent cannot be assessed based on a single evaluation [28,29], hence a number of methods with different mechanisms have been employed in this study. The models used for the determination of antioxidant potentials of the LLFPME were DPPH and hydrogen peroxide scavenging activities, metal chelating, total antioxidant capacity and ferric reducing antioxidant power.

The potent antioxidant activities reported in this study may be attributable to the substantial presence of total phenolic and flavonoids in the LLFPME. The antioxidants act through the transfer of hydrogen atoms and by transfer of electrons or chelating of metals [29,30]. The LLFPME showed strong DPPH scavenging activity, ferric reducing antioxidant power and total antioxidant capacity; which compared favourably with the standard drugs used and are consistent with earlier studies [28,29,31,32]. Olarewaju*et al.*[32] reported that total antioxidant capacity of a vegetable is primarily due to the presence of flavonoids

and related polyphenols while ferric reducing antioxidant power of selected plant leaves were dose dependent.Fajobi*et al*[29] and Morakinyo*et al.* [31]also reported dose dependent activities of *Pterocarpusmildbraedii*and *Aframomummelegueta*leaves respectively on DPPH and H_2O_2 radicals, TAC and FRAP.

Transition metal ions, putting aside their goods, are liable to cause peroxidation of unsaturated fatty acid and contribute to the oxidative damage in some diseases such as Alzheimer's and Parkinson's diseases[33]. Also, the condition referred to as metal toxicity or poisoning can only be treated using metal chelating therapy in which drugs like dimercaptosuccinic acid, EDTA, deferoxamine and dimercaprol are prescribed for the patient [34]. Considering the importance of metal in biological systems, metal chelating activity of the extract was tested using ferrozine as a chelating agent. In this assay, decolorization of Fe^{2+}-ferrozine complex takes place due to the presence of chelating agents in the LLFPME. The result acquired showed no significant difference from the EDTA used as the standard drug. It implied that the extract could be employed in the treatment of metal toxicity.

Inflammation is a typical dynamic reaction to stimuli that cause tissue damage, such as heat, chemicals, and microbial infections [35,36]. Inflammation is the body's defensive mechanism to remove harmful stimuli and start the tissue-healing process [35]. Anti-inflammatory plant extracts have the ability to maintain cell membrane integrity against lysis, prevent protein denaturation and inhibit lipoxygenases [37], thus facilitating the stability of biological membrane when stressed. Cell viability is reliant on the integrity of their membranes [38], and when red blood cells are damaged by heat or hypotonic medium, their membranes lyses, a condition known as hemolysis [39]. Erythrocyte membrane stabilization, analogues of lysosomal membranes are crucial for mimicking the inflammatory responses because it prevents the release of bactericidal and protease enzymes, which are lysosomal constituents of activated neutrophils that cause more tissue damage. Damage to the erythrocyte membrane increases the cell's susceptibility to secondary damage from lipid peroxidation by free radicals. During appropriates stimulation of neutrophils, arachidonic acid is cleave from phospholipids membrane and converted into leukotrienes and prostaglandins through the lipoxygenase and cyclooxygenase pathways, respectively, this is one of the process of anti-inflammatory response. Leukotrienes, a crucial mediator in a range of inflammatory processes, are produced when lipoxygenase catalyzes the oxidation of arachidonic acid

[40]. Therefore, *in-vitro* lipoxygenaseinhibition acts as an excellent model in testing plants with anti-inflammatory properties. The anti-inflammatory activities of LLFPME showed the inhibition of lipoxygenase activity which might be due to the presence of bioactive components including 9-Octadecenoic acid (Z)-methyl ester reported to inhibit lipoxygenase activity [23,24]. The LLFPME anti-inflammatory activity was also substantiated by stabilization of the red cell membrane against hypotonic and heat induced lysis. The results obtained in this study are in agreement with earlier studies [17,41] that reported the membrane stabilizing effect of *Theobroma cacao* root extract on red blood cell challenged with heat and hypotonic stress, and inhibitory activities of selected plants on lipoxygenase activity, respectively.

The anti-bacterial properties of LLFPME exhibited in this study might be attributed to the fatty acids components present in *L. leucocephala*, which causes disruption to the electron transport chain as well as the oxidative phosphorylation and membrane enzymes responsible for energy production in bacteria. These disruptions lead to loss of bacteria membrane integrity, lysis and death [24,42].The LLFPME showed zone of inhibition against *Clostridium sporogens*, *Bacillus cereus,Klebsiella pneumonia*, *Bacillus stearothermophillus*, *Bacillus subtilis* and *Bacillus cereus* at 20 mg/ml concentration, minimum inhibitory concentration at range of 1.25 and 10 mg/ml, and minimum bactericidal concentration at range of 5 and 20 mg/ml. Obiang*et al.*[30] reported that the inhibition of bacteria growth might be due to the presence of phenolic compounds in plant extracts.It has also been reported that phenolic compounds extracted from plant inhibit bacterial growth by inducing morphological changes and cytoplasmic leakage [24,43]. Obiang*et al*. [30] also reported that the antimicrobial properties of a plant could be attributed to their fatty acid and phenolic contents, which were revealed by GCMS analysis to be present in the LLFPME. The results observed in this study were similar to other studies [2,24,42,44] that reported the concentration dependent inhibition of bacteria growth of *Terminalia avicenniodes*and *Terminalia superba*.Our study showed that the plant pod has bioactive principles that can be investigated for the management and treatment of infections, as evidenced by the observed antibacterial capability. The majority of the bacteria that showed sensitivity to the extract from the plant pod caused diseases that have recently raised concerns for public health [45-48]. For instance, the World Health Organization has designated

*Klebsiellapnaeumoniae*as a pathogen of concern for public health, placing it in the critical priority category of diseases causing pathogens[49].

5. CONCLUSION

It was concluded that LLFPME exhibited antioxidant potential through various mechanisms, which possibly aided its ability to stabilize stressed cell membrane and similarly inhibited the growth of some bacterial strain. These observed antioxidant, anti-inflammatory and anti-bacterial activities might be due to the bioactive compounds present in the plant part.

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