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Original Research Article

Antimalarial activity of *Mangifera indica* aqueous extract in Plasmodium berghei's apicoplast

Edet E Asanga[1]*, Henshaw Okoroiwu[2], Uwem O Edet[3], Dennis Amaechi[4], Promise E Nelson[1], Mercy Uchenwa[5], Olorunfemi A Eseyin[6], Goodnews Samuel[7], Lordswill A Ettah[1], Osuake A Obongha[1]

[1]Department of Biochemistry, [2]Department of Medical Laboratory Science, [3]Department of Microbiology, Arthur Jarvis University, [4]Department of Biochemistry, Veritas University, Abuja, [5]Department of Microbiology, University of Calabar, [6]Department of Pharmacognosy and Natural Medicine, University of Uyo, [7]Department of Chemistry, Arthur Jarvis University, Calabar, Nigeria

For correspondence:* ***Email:* *edyasangae1@gmail.com;* ***Tel:*** *+234-8032586166*

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Abstract

Purpose: *To investigate the in vivo antiplasmodial effect of Mangifera indica aqueous extract in Plasmodium berghei-infected mice, and to characterize its phytochemical constituents and their mechanism of action.*

Methods: *The plant leaves (250.71 g) and stem bark (509.34 g) were weighed, macerated in 5 L of boiled, distilled water for 72 h, and filtered. In vivo antiplasmodial evaluation of the extract was carried out using Plasmodium berghei-infected mice. The leaf and stem bark extracts were subjected to gas chromatography-mass spectroscopy (GC-MS) analysis for characterization of their chemical constituents.*

Results: *The leaf/stem bark (1:2) extract (88.27 %) exhibited chemosuppressive activities that was better than artesunate (88.04 %) and artemisinin-based combination therapy (ACT, 84.60 %). Its schizonticidal activities (98.63 %) revealed a better therapeutic response when compared with artesunate (92.34 %). Spectroscopic analysis showed linoleic acid esters and other compounds.*

Conclusion: *Therapeutic response of leaf/stem bark (1:2) extract exhibit superior schizonticidal activity to ACT and artesunate. Furthermore, the linoleic acid esters in Mangifera indica aqueous extract may contribute to strong antimalarial activity by inhibiting fatty acid biosynthesis.*

Keywords: *Mangifera indica, Antiplasmodial, Linoleic esters, Herbal formulation*

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INTRODUCTION

Malaria is a terrible disease that affects almost 0.5 percent of the world's population [1]. A study by a group of scientists [2] revealed that about 40 percent of human race is likely to contract malaria; half of this population is resident in Africa, with almost 80 percent of such cases found in Nigeria, Tanzania, Congo, Kenya, and Ethiopia [2]. Malaria is caused by plasmodium parasite, and there are reports of resistance by the parasite against chemotherapies like

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artesunate and ACT [3], and this is a limitation to malaria prevention and elimination in Nigeria. Over the years, phytomedicines have been reported as an alternative in the management of malaria in some countries. *Mangifera indica* (mango) is a pharmacologically rich and diverse plant [4]. Some of its reported pharmacological potentials are anti-inflammatory, antiplasmodial, and antioxidant in different parts [4]. Many metabolites have been isolated from the plants using different techniques, although some of their mechanisms of action are unknown.

Fatty acids are one of the metabolites present in plants. They inhibit biosynthesis in the *Plasmodium* parasite through the alteration of metabolic activities in the parasites' apicoplasts [5]. The apicoplast is where the generation of isoprenoids through the mevalonate-independent 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway takes place [5]. This pathway is very essential in the growth of malaria parasites. Moreover, polyunsaturated fatty acid (PUFA) is implicated in the inhibition of fatty acid biosynthetic machinery of *Plasmodium* parasite [6]. While linoleic and linolenic acids have medicinal properties, n-3 and n-6 PUFA have antimalarial properties.

However, antiplasmodial activity of the herbal formulation of *Mangifera indica* administered to *Plasmodium berghei*-infected mice was compared with that of artesunate and ACT. Its antiplasmodial constituent was characterized and their possible mechanisms of action was determined.

EXPERIMENTAL

Sourcing and authentication

The stem bark and leaves of *Mangifera indica* were obtained in April 2021 from Arthur Jarvis University, Nigeria. The plant parts' identity was determined by their ascertained number, UCH432G, which was in the department's herbarium after authentication by Taxonomist Dr. Godwin Eteng.

Processing of herbal formulation

The plant parts were sorted, washed, and dried. A mortar and pestle were used to pulverize the plant parts into coarse particles. The dried coarse plant materials were subsequently divided into groups consisting of leaves (250.71 g), stem bark (509.34 g), leaves/stem bark (1:1) (101.24/101.24 g), leaves/stem bark (1:2) (164.39/328.78 g), and leaves/stem bark (2:1) (218.50/109.25 g). Each group was macerated in boiled distilled water (5 L), shaken occasionally within 72 h, filtered with sieve cloth and Whatman filter paper, and concentrated with the aid of a rotary evaporator (at 40 ^{o}C) to get the various aqueous extracts. The weights and % yields for each extract were: 31.3 g leaves (15.2 %), 43.8 g stem bark (8.7 %), 34.8 g leaves/stem bark (1:1) (17.2 %), 29.2 g leaves/stem bark (1:2) (5.92 %), and 26.1 g leaves/stem bark (2:1) (7.96 %). The herbal formulation of infused aqueous extracts was preserved and used for the study.

Animal handling

One hundred and sixty-five Wistar (13 – 27 g) mice of both sexes were sourced from the Animal House, Department of Pharmacology and Toxicology, University of Uyo, Nigeria, and used for the antiplasmodial study. The NIH guideline for handling and usage of experimental animals (the ARRIVE guidelines in compliance with NIH publications no. 8023, revised 1978) was judiciously followed. The mice were given the freedom to access a standard pellet diet, water, and were housed at ambient temperature. The Animal Ethics Committee of the Faculty of Pharmacy, University of Uyo, Nigeria approved the study (approval no. UUFPHARM/01933) and ensured strict compliance with the experimental protocols.

Determination of acute toxicity

Lethal dose (LD_{50}) and effective doses (ED_{50}) were calculated by using Lorke's method [7] which involved phases I and II. Rats were observed for 24 hours while the number of deaths were recorded in order to establish the medial dosage of the herbal formulation of the plant as well as the one that might induce toxicity in a short term in mice.

Parasites

The National Institute of Medical Research (NIMR), Nigeria, provided the *Plasmodium berghei* (NK-65) infected donor mice used in this study, and mice were acclimatized according to the method described by some researchers [8].

Inoculum preparation

The inoculum of the parasite-infected erythrocytes with about 0.2 mL peripheral parasitemia was obtained from the donor mice via cardiac puncture and emptied into an anti-coagulant-coated tube. Percentage of parasites in blood and concentration of the original inoculum were calculated according to the method described by some researchers [9].

Drug administration

Antiplasmodial screening followed the dissolution of tablets of ACT (560 mg) in distilled water (100 mL) and was given orally at doses of 8 mg/kg body weight of experimental animals, whereas that of artesunate (100 mg) was orally given at doses of 5 mg/kg body weight of mice after dissolution in 20 mL of distilled water.

In vivo antiplasmodial determination

A method by Asanga *et al* [8] was slightly modified and used for the evaluation of *in vivo* antimalarial properties of herbal formulation of the plant.

Microscopic examination

Each slide was prepared by measuring blood (2 µL) for the thin film as well as 6 µL for the thick film according to an earlier report [8], whereas their parasite densities and % growth inhibition of *Plasmodium berghei* were calculated and expressed as earlier reported by [8,10].

Gas chromatography-mass spectroscopy (GC-MS)

The extracts of *Mangifera indica's* herbal formulation were each weighed (10 mg), dissolved in DMSO, and subjected to GC-MS analysis according to the method described by [8,11]. Two (2) µL (split ratio 10:1; split flow 12 mL/min) was injected into an Agilent system consisting of a model 7890 N gas chromatograph and a model mass detector Triple Quad 7000 A in EI model at 70 eV (m/z range 40 – 600 amu; Agilent Technologies, Santa Clara, California. USA.

GC column was an HP-5ms fused silica capillary with a 5 % phenyl-methyl polysiloxane stationary phase (30 m x 250 µm x 0.25 µm). The carrier gas was helium with a column head pressure of 9.7853 psi and flow rate of 1.2 mL/min. Inlet temperature was 250 ^{o}C and mass selective detector temperature was 250 ^{o}C.

The GC oven temperature programming used was as follows: 50 ^{o}C initial temperature was held for 10 minutes; increased at 6 ^{o}C/min to 190 ^{o}C for 20 minutes; increased 7 ^{o}C/min to 290 ^{o}C for 30 minutes. Compounds were identified based on their retention indices and by comparing their mass spectral fragmentation patterns with the National Institute of Standards and Technology (NIST) database/ChemStation data system.

Statistical analysis

GraphPad Prism version 9.0 (GraphPad Software, Incorporated, CA, USA) was used to analyze the statistics and presented as mean ± standard error of the mean (SEM). The differences that were significant within and between the different treatment groups were analyzed using one-way ANOVA, and then their means were compared using Turkey's *post hoc* test. Differences at a confidence level of 95 % were considered to be statistically significant.

RESULTS

The results (Table 1) highlighted the order of % growth inhibition of parasites as follows: ACT (77.64 %) > leaves/stem bark (1:2) (76.20 %) > stem bark + artesunate (72.47 %) > leaves + artesunate (70.54 %) > stem bark (68.61 %) > leaves (67.90 %) > artesunate (63.87 %) > leaves/stem bark (1:1) + artesunate (58.15 %) > leaves/stem bark (2:1) (54.21 %) > leaves/stem bark (1:1) (53.42 %) > negative control.

The chemosuppressive efficacy of the drugs and *Mangifera indica's* herbal formulation are presented in Table 2. The decreasing order of growth inhibition of the parasite was as follows: leaves/stem bark (1:2; 88.27 %) > artesunate (88.04 %) > ACT (84.60 %) > stem bark + artesunate (81.56 %) > leaves + artesunate (79.30 %) > stem bark (76.65 %) > leaves/stem bark (1:1) + artesunate (66.20 %) > leaves/stem bark (2:1) (51.46 %) > leaves (41.82 %) > leaves/stem bark (1:1) (33.02 %).

Table 3 shows the schizonticidal activities of *Mangifera indica*'s herbal formulation when compared with negative control groups, ACT, and artesunate groups to establish their pharmacological activities. Parasites' growth inhibition on day 5 (D5), in decreasing order, were as follows: ACT (98.92 %) > leaves/stem bark (1:2) (98.63 %) > stem bark + artesunate (97.08 %) > leaves/stem bark (1:1) + artesunate (95.41 %) > leaves + artesunate (95.24 %) > stem bark (95.20 %) > artesunate (92.34 %) > leaves/stem bark (1:1) (91.10 %) > leaves/stem bark (2:1) (86.04 %) > leaves (81.02 %) > negative control (-136 %).

Results from GC-MS spectra showed the presence of many compounds with different retention times and percentage abundance (area, %), as illustrated in Table 4 and Table 5. Mass spectrometer (MS) analyzed the eluted compounds at varying times to characterize their chemical structures, nature,

Table 1: *In vivo* antiplasmodial activity (prophylactic test model, n = 5)

Treatment group	Mean weight (g)	Parasite density/µL, growth inhibition (%)	Mean survival time (MST, days)
Negative control	22.8±1.66	902986±48631	11.6±0.68
Artesunate (5mg/kg)	34.2±1.66	326214±51846 (63.87%) [a]*	21.8±0.80[a]**
ACT (5mg/kg)	23.8±0.86	201881±100691 (77.64%) [a]*	25.2±0.37[a]**
Leaves (300mg/kg)	22.6±1.29	289875±37533 (67.90%) [a]*	18.0±1.34[a]**[b]*[c]*
Stem bark (300mg/kg)	23.0±1.36	283463±37533 (68.61%) [a]*	22.0±0.55[a]**
Leaves/stem bark (1:1)	23.8±1.59	420647±70107 (53.42%) [a]*	19.22±0.49 [a]** [c]*
Leaves/stem bark (1:2)	23.4±1.44	248549±17068 (76.20%) [a]*	20.0±0.45 [a]** [c]*
Leaves/stem bark (2:1)	22.6±1.93	413444±45139 (54.21%) [a]*	19.2±0.49 [a]** [c]*
Leaves/artesunate	23.0±1.79	265940±36677 (70.54%) [a]*	19.0±0.45 [a]** [c]*
Stem bark/artesunate	22.4±1.08	214950±49505 (72.47%) [a]*	17.0±0.45 [a]** [b]*[c]*
Leaves/stem bark (1:1)/artesunate	21.6±1.40	377933±40126 (58.15%) [a]*	17.6±1.12 [a]** [b]*[c]*

[a]*Significant decrease, $p < 0.05$ versus control; [a]**significant increase, $p < 0.05$ versus control; [b]*significant decrease, $p < 0.05$ versus artesunate; [c]*significant decrease, $p < 0.05$ versus ACT

Table 2: *In vivo* antiplasmodial (suppressive test model, n = 5)

Treatment group	Mean weight (g)	Parasite densities/µL, growth inhibition (%)	Mean survival time (MST) (days)
Negative control	17.8±01.16	981109±246701	9.40±0.25
Artesunate (5mg/kg)	16.8±1.16	117364±55408 (88.04%) [a]*	19.8±1.46[a]**
ACT (5mg/kg)	17.8±0.2	151131±19953 (84.60%) [a]*	22.2±1.93[a]**
Leaves (300mg/kg)	17.8±1.16	570785±126611 (41.82%) [a]*	13.40±1.25
Stem bark (300mg/kg)	16.8±0.37	230565±88940 (76.65%) [a]*	14.00±1.14[a]**
Leaves/stem bark (1:1)	17.4±1.29	657107±180561 (33.02%)	16.00±1.41
Leaves/stem bark (1:2)	18.2±1.36	124918±27679 (88.27%) [a]*	20.00±1.73[a]**
Leaves/stem bark (2:1)	17.4±0.25	476266±279023 (51.46%)	14.6±03
Leaves/artesunate	18.8±1.59	203083±24764 (79.30%) [a]*	23.4±1.50[a]**
Stem bark/artesunate	16.8±1.36	180953±67647 (81.56%) [a]*	22.4±1.69[a]**
Leaves/stem bark (1:1)/ Artesunate	18.0±0.45	331591±65331 (66.20%) [a]*	22.0±1.41[a]**

[a]*Significant decrease, $p < 0.05$ versus control; [a]**significant increases, $p < 0.05$ versus control

and retention times. Appearance of peaks at different m/z ratios arose from the fragmentation of large compounds into smaller fragments. The fingerprint of compounds is characterized by a mass Hunter data library system. The GC-MS analysis of plant extract showed that the most abundant compounds were 9,12-octadecadienoic acid, oleic acid, 9,12-octadecadienal, 9-oxabicyclo (6.1.0) nonane, hexadecadienal, and spirohexane.

DISCUSSION

Repository assay of drugs is very critical in antiplasmodial research. The results showed that the parasite densities were significantly reduced in the entire group as compared with the negative control group; therefore, all the treatment groups had remarkable repository activity against malaria parasites, nevertheless, with varying capacities. Increased inhibition (%) of parasitemia in this study was consistent with an earlier report [12]. *Plasmodium berghei* inhibition (%) by leaves and stem bark (1:2), stem bark, and leaves had better repository activities than artesunate. However, this could be due to the linoleic acid esters in the extract, as some researchers posited that the linolenic and linoleic acids were more potent against *P. berghei* in mice [5].

Furthermore, the prophylactic potentials of *Mangifera indica*'s herbal formulation are indicative of their potential in the destruction of the various stages of the parasite, whereas some drugs' mechanisms of action involve the destruction of the first stage of parasite development in the hepatic tissue or the suppression of the emerging asexual stages, as well as the destruction of hypnozoites to avert relapses [13]. Thus, the consumption of

Table 3: *In vivo* antiplasmodial (curative test model, n = 6)

Treatment group	Weight (g)	Parasite densities/ µL on day-1, % growth inhibition	Parasite densities/ µL on day-3, % growth inhibition	Parasite densities/ µL on day-5, % growth inhibition	Mean survival time (MST) (days)
Negative control	21.8±2.27	501227±86808	751789±66770 (-50.00%)	1183193±91244 (-136.1%)[a]*	10.0±0.32
Artesunate (5mg/kg)	24.8±1.8	505041±1932455	90666±46231 (82.05%)[a]*	38975±15927 (92.34%)[a]**	25.4±0.68[a]**
ACT (5mg/kg)	21.8±2.44	893055±248644	17410±2340 (98.05%)[a]*	9618±3845 (98.92%)[a]*	24.0±2.55[a]**
Leaves (300mg/kg)	22.8±1.77	763622±131693	272337±47298 (64.23%)[a]*	143574±41595 (81.02%)[a]*	17.2±1.66[a]**
Stem bark (300mg/kg)	22.8±1.16	1109288±445783	146039±51047 (87.06%)[a]*	57228±21744 (95.20%)[a]*	21.0±1.76[a]**
Leaves/stem bark (1:1)	21.2±0.86	946575±144486	154651±20098 (84.40%)[a]*	81161±16605 (91.10%)[a]*	13.4±0.25
Leaves/stem bark (1:2)	23.2±1.32	987066±100166	85552±21733 (90.06%)[a]*	12241±2073 (98.63%)[a]*	23.8±1.59[a]**
Leaves/stem bark (2:1)	23.8±2.13	733286±241403	212864±58273 (71.32%)[a]*	105702±11567 (86.04%)[a]*	11.4±0.51
Leaves/ artesunate	22.2±2.15	996323±129989	135125±31121 (86.17%)[a]*	49991±14959 (95.30%)[a]*	20.4±1.29[a]**
Stem bark/ artesunate	24.4±1.89	978022±55214	71567±29551 (93.42)[a]*	29435±15442 (97.08)[a]*	21.6±1.94[a]**
Leaves/stem bark (1:1)/ Artesunate	23.8±2.06	754377±107246	68360±27602 (90.94)[a]*	34500±9815 (95.41)[a]*	19.4±1.21[a]**

[a]*Significant decrease, $p < 0.05$ versus control; [a]**significant increases, $p < 0.05$ versus control

Table 4: GCMS analysis of the extract of *Mangifera indica* stem bark

Compound	Mean area (%)	Retention time (min)
9-Oxabicyclo (6.1.0) nonane hypoxanthine	0.05	5.624
Shikimic acid L-Galactose, 6-deoxy- 2-Formyl-9-(beta.-d-ribofuranosyl)	0.70	5.932
Z-8-Methyl-9-tetradecenoic acid, Oxacyclotetradecan-2-one	1.77	6.404
N-ethyl- Undec-10-ynoic acid, dodecyl ester	1.32	6.747
9,12-Octadecadienoyl chloride,	0.83	6.981
7,11-Hexadecadienal, cis-7-Dodecen-1-ol	0.11	8.006
9,12- Octadecadienal	0.44	8.254
9-Oxabicyclo (6.1.0) nonane, cis-	2.69	8.615
9,12-Octadecadienoic acid ester	0.07	9.401
Succinic acid, tridec-2-yn-1-ylbut-2-en-1-yl este	0.18	9.866
9-Oxabicyclo (6.1.0) nonane, cis-	0.05	10.286
9,12-Octadecadienoyl chloride dodecyl	0.09	10.857
9,17-Octadecadienal	0.09	11.130
9,12-Octadecadienoyl	0.07	11.340
Cyclopentane undecanoic acid	0.15	11.502
2-Methyl-Z,Z-3,13-octadecadienol	0.09	11.664
Pentanoic acid, 10-undecenyl ester; Oleic Acid	0.09	11.845
7,10-Hexadeca Dienoic acid, methyl ester	6.95	12.968
9,12-Octadecadienoic acid, methyl	1.71	13.317
Cyclopentadecanol	4.19	13.752
Oleic Acid; 9,12-Octadecadienal	2.21	14.261
2-Methyl-Z,Z-3,13-octadecadienol	1.79	14.409
9,12-Octadecadien-1-ol, (Z,Z)-	2.76	14.967
9,12-Octadecadienal Oleic Acid	0.98	15.240
9,12-Octadecadienal	1.66	15.458
9,12-Octadecadienal	1.08	15.660
Oleic acid	3.21	15.971
9,12-Octadecadienal; Oleic acid	0.82	16.439

Table 4: GCMS analysis of the extract of *Mangifera indica* stem bark (*cont'd*)

Compound	Mean area (%)	Retention time (min)
9,12-Octadecadienal	0.65	16.597
13Oxabicyclo (10.1.0) tridecane	1.79	16.732
9,12-Octadecadienal; Oleic acid	1.40	17.172
9,12-Octadecadienal; 9,12-Octadecadienoic acid (Z,Z)	0.94	17.292
Oleic acid 9,12-Octadecadien-1-ol, (Z,Z)-	1.28	17.565
9,12-Octadecadienoic acid (Z,Z	1.73	17.771
9,12-Octadecadienal	0.78	17.986
9,12-Octadecadienoic acid, methyl ester, (E,E)-	2.06	18.153
9,12-Octadecadienal	2.72	18.498
9,12-Octadecadienal; 1,6-Octadiene, 3,7-dimethyl-, (S)	1.64	18.755
9,12-Octadecadienal; 4-Pentenal, 2-methylene-E-1,6-Undecadiene	1.07	18.996
Dichloroacetic acid, undec-2-enyl ester; 9,12-Octadecadienal	0.75	19.172
9,12-Octadecadienal; Cyclohexene, 4-(4-ethylcyclohexyl);	1.44	19.304
9,12-Octadecadienal; Cyclopentaneundecanoic acid; 2-Methyl-E,E-	1.98	19.595
Oleic acid; 9,17-Octadecadienal, (Z)-	1.23	19.921
9,12-Octadecadienal; Oleic acid; 9-Oxa Bicyclo (6.1.0) nonane	1.86	20.153
9,12-Octadecadienal	0.64	20.449
Oleic acid; 9,12-Octadecadienal	1.15	20.578
1,11-Dodecadiene; 9,17-Octadecadienal, (Z)-	2.32	20.899
9,12-Octadecadienal	1.86	21.221
3,13-octadecadien-1-o	0.76	21.579
trans-13-Octadecenoic acid; (S)(+)-Z-13-Methyl-11-	1.82	21.738
9,12-Octadecadienal; 2-Methyl-E,E-3,13-octadecadiene-	1.80	22.104
9,12-Octadecadienal; 3-Octyne, 6-methyl-; 9-Oxabicyclo (6.1.0) nonane	0.59	22.494
9,12-Octadecadienal; Oleic Acid; 9-Oxabicyclo (6.1.0) nonane, cis-	1.17	22.679
9,12-Octadecadienal cis-7, cis-11-	1.25	22.907
9,12-Octadecadien-1-ol, (Z,Z)-	0.58	23.143
undec-2-enyl ester;13-Octadecenal, (Z)	0.70	23.262
1,6-Octadiene, -3,7-dimethyl-, (S)-; 9,12-Octadecadienal; Oleic acid	1.75	23.455
9,17-Octadecadienal, (Z)-	0.75	23.745
9,12-Octadecadienal; 1,6-Octadiene, 3,7-dimethyl-	0.69	0.69
9,12-Octadecadienal; Dichloroacetic acid, undec-2-enyl ester; Oleic acid	2.40	24.402
Spirohexan-4-one, 5,5-dimethyl-; Oxonin, 4,5,6,7-tetrahydro-	4.48	25.116
Cyclohexene, 1,2-dimethyl-; 9,12-Octadecadienal; 3-	4.57	25.244
1,6-Octadiene, 3,7-dimethyl-, (S)-; 9,12-Octadecadienoyl chloride	1.02	25.912
9,12-Octadecadienal	0.85	26.220
7,11-Hexadecadienal	0.37	26.393
Oleic acid; 9,12-Octadecadienal; Cyclopentane undecanoic acid	0.59	26.544
1,6-Octadiene, 3,7-dimethyl-, (S)- 9,12	0.68	26.675
9,12-Octadecadienoic acid	0.28	27.436
1-Cyclohexyl nonene; Cyclohexene, 4-(4-ethylcyclohexyl) -1-pentylcis-	0.45	27.720
1,6-Octadiene, 3,7-dimethyl-	0.24	27.927
1,12-Dodecanediol; cis-9,10-Epoxy octadecan-1-ol; Oleic acid	1.29	28.397
12-Octadecadienal; 7,11-Hexadecadienal	0.25	28.617
9,12-Octadecadienal; 9,12-Octadecadienoic acid, methyl ester	0.64	28.786
9,12-Octadecadienal	0.30	29.399
Spirohexan-4-one, 5,5-dimethyl-	0.50	29.695
9,12-Octadecadienoic acid; 9-Octadecenoic acid Oleic Acid	0.42	29.975
3-Octyne, 6-methyl	0.57	30.079
3-Octyne, 6-methyl; 1,12-Dodecanediol	0.22	30.556
9-Oxabicyclo (6.1.0) nonane	0.33	30.981
Cyclopentane undecanoic acid	0.14	31.170
9,12-Octadecadienal; 9,12-Octadecadienoic acid	0.12	31.310

Table 5: GCMS analysis of an aqueous extract of *Mangifera indica* leaves

Retention time (min)	Mean area (%)	Compound
5.6395	0.1614	2-Hexyn-1-ol
5.8575	0.9748	2-Hexyn-1-ol
6.1887	0.1526	1-(p-Toluidino)-1-deoxy-.beta.-d-Idopyranose
6.3593	0.283	Eicosyl propyl ether
7.0018	0.3839	Morpholine, 4-methyl-, 4-oxide
7.4801	0.7945	2-Hexyne
7.7528	2.1343	Dodecanoic acid
8.0478	0.9236	9,12-Octadecadienal
8.2897	1.6572	9,12-Octadecadienal
8.6533	5.5199	9,12-Octadecadienal
13.064	12.1682	9-xabicyclo (6.1.0) nonane
13.3407	3.0178	2-Methyl-E,E-3,13-octadecadien-1-ol
13.6912	2.7701	9,17-Octadecadienal, (Z)-
14.1224	4.3057	9,12-Octadecadienal
14.2963	2.2704	9,12-Octadecadienoic acid, methyl ester, (E, E)-
14.5514	5.8698	Oleic Acid
14.9751	2.0471	9,12-Octadecadienal
15.174	1.8319	9,12-Octadecadienal
15.3986	1.7522	Oleic Acid
16.367	4.6382	9,12-Octadecadienoic acid, methyl ester, (E, E)-
17.1285	2.833	Oleic Acid
17.8908	1.2885	9,12-Octadecadienal
18.2009	2.8251	9,12-Octadecadienal
18.473	1.1179	Oleic Acid
19.5623	4.3558	9-Oxabicyclo (6.1.0) nonane
23.2022	0.7322	9-Oxabicyclo (6.1.0) nonane, cis-
23.3099	0.5021	9-Oxabicyclo (6.1.0) nonane
23.459	1.2027	9,12-Octadecadienal
23.8257	1.1491	cis-13-Octadecenoic acid
25.0103	7.5301	9,12-Octadecadienal
25.6198	12.6493	9,12-Octadecadienal
26.1518	1.7604	9,12-Octadecadienoic acid (Z,Z)-
26.4306	1.0297	1,14-Tetradecanediol

Mangifera indica's herbal formulation before the manifestation of the clinical symptoms of malaria may help to attenuate malaria incidence.

Mean survival time (MST) is a pharmacological parameter used in the evaluation of drug efficacy; it gives clues on the parasitemia clearance time in the host system. The longer the MST of a drug, the higher its therapeutic response; hence, the better its efficacy. The significant increase in MST of the entire groups treated with *Mangifera indica's* herbal formulation when compared to the negative control suggests that the plant has good repository activity against malaria infection. Nevertheless, with respect to MST, it was only the stem bark (300 mg/kg) of this plant that compared favourably with that of ACT. This suggests a similarity in the metabolites of this extract to the compounds that enhance parasitemia clearance and recovery from malaria infection. The chemosuppressive potential of the herbal formulation (Table 2) showed that all the treatment models produced significant reductions in their parasite densities as compared with the group of negative controls; hence, the extracts greatly suppressed *Plasmodium berghei*-induced infection in the animal model used. Therefore, the chemosuppressive activity of the herbal formulations against the parasite could be attributed to the esters of linoleic acid present in the plant. Some researchers [13,14] reported that some plant extracts exert antimalarial action via the mitigation of fatty acid and protein biosynthesis in the parasites.

The results on the significant increases in % growth inhibition of plasmodium parasites in all the treatment models as compared with the group of negative controls are an indication that the herbal formulation had excellent chemosuppressive potentials and were in tandem with an earlier report by some researchers [14]. Consequently, the chemosuppressive abilities against malaria infection of the leaves and stem bark (1:2) and

stem bark/artesunate compared favourably with artesunate and ACT, respectively.

Furthermore, all the groups except leaf, leaves/stem bark (1:1), and leaf/stem bark (2:1) showed significant increases in their MST as compared with the group of the negative control. Therefore, the results of the above-listed treatment groups corroborated their growth inhibition of parasites as well as parasite densities, suggesting they were not effective in parasitemia clearance. However, other treatment groups were remarkable in malaria treatment.

In addition, most antimalarial drugs' mechanisms of action target the inhibition of the growth and replication of *Plasmodium* parasites. The increase in growth inhibition of the malaria parasite in all the groups as compared with the group of negative controls were consistent with an earlier report by some researchers [8] on *Nauclea latifolia* root extract and fractions (150 mg/kg). Kumaratilake *et al* [15] reported that fatty acids induce direct destruction of malaria parasites, whereas others act through neutrophil priming. Therefore, the presence of esters of linoleic acid and other compounds in this herbal formulation may have aided the destruction of parasites. More so, the role of long-chain fatty acids in the inhibition of *P. berghei's* enoyl-ACP reductase (FabI), an enzyme that catalyzes the fatty acid elongation cycle in *P. berghei* was reported [16]. Therefore, the mechanism of action of *Mangifera indica's* herbal formulation may be as result of the inhibition of the biosynthesis of fatty acids in the parasites' apicoplast due to the esters of linoleic acid. A similar finding was reported by some scientists

[8] on the significant increases in MST in mice where it was proposed that the prolonged MST for the parasitized mice resulted from higher parasitemia clearance by the herbal formulation, leading to higher schizonticidal activities.

CONCLUSION

There is a significant therapeutic response to leaves and stem bark (1:2) extracts against *Plasmodium berghei*-induced malaria in mice, as corroborated by its stronger repository, chemosuppressive, and schizonticidal activities than ACT and artesunate. Moreover, linoleic acid esters, shown to be present by GC-MS data, have previously been reported to act through the inhibition of fatty acid synthase type II and enzymes of mevalonate-independent 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway in *Plasmodium berghei's* apicoplast.

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Ethical approval

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Edet E Asanga, Henshaw Okoroiwu, Uwem O Edet, Dennis Amaechi, and Promise E Edem, conceived the study and carried out data analysis. All authors were involved in the drafting and approval of the manuscript for final publication.

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