Original Research Article

Antimalarial Assay, Isolation, and Characterization of Compounds Responsible for Antimalarial Activity in Danielliaoliveri (Rolfe) Hutch.& Dalziel (Caesalpiniaceae).

Abstract

Aim: The research aimed to investigate the antimalarial activity of the plant *Danielliaoliveri* (Rolfe) Hutch. & Dalziel (Caesalpiniaceae) bark methanol extract.

Methodology: The bark dried, powdered bark of *Danielliaoliveri*was extracted with n-hexane followed by methanol. methanol extract of *Danielliaoliveri* was tested for antimalarial (curative test) by being tested against Plasmodium berghei in mice at doses of 100, 400, and 600 mg/kg. An acute toxicity study was carried out according to OECD 423 protocol, and phytochemical screening using standard protocols. HPLC-MSwas also conducted using Agilent InfinityLab LC/MSD with Eclipse plus C18 5.0µm 4.6mm x 250 mm column and data were processed with Agilent OpenlabChemstation software.

Results: Preliminary phytochemical screening of the extract revealed the presence of flavonoids, tannins, alkaloids, phenolics, and terpenoids. The methanol extract was foundto be safe at doses up to 5000 mg/kg body weight (acute lethal dose, LD50) in mice. There was a significant difference in activity between the non-treated, standard, and extract dosage groups at ($p<0.05$). The HPLC-MS analysis showed the presence of 56 compounds. *Daniellaoliveri* bark methanol extract has High activity against Plasmodium berghei, an 81.1% reduction in parasitemia at a dose of 600mg/kg body weight, and a Mean survival time of excess 5.

Conclusion: *Danielliaoliveri* is a rich source of phytochemicals and a potential source of antimalarial agent(s)

Keywords: *Danielliaoliveri*; Malaria; *Plasmodium*; ACT; HPLC-MS; phytochemistry

Introduction

"Malaria is a life-threatening disease caused by the infection of red blood cells with protozoan parasites of the genus Plasmodium that are transmitted to people through the bites of infected female Anopheles mosquitoes. Four species of Plasmodium (*P. falciparum, P. vivax, P. malariae,* and *P. ovale*) most commonly infect humans. *P. falciparum* and *P. vivax* are the most prevalent species and P. falciparum is the most dangerous" [1]. "In 2020, malaria caused 627 000 estimated death" [2]. "Malaria is preventable and treatable, and the global priority is to reduce the burden of disease and death while retaining the long-term vision of malaria eradication" [1]. Since the identification of malaria as a disease and the mosquito as a vector, many agents have been employed in the treatment of the disease and/or the eradication of the disease. Manyagents both natural and synthetic have been engaged in the war against malaria but the parasite has fought back through the development of resistance to these agents. One compound that has stood out for which there is no resistance is quinine from *Cinchona officinalis.* Toxic side effects restrict its regular use. Artemisinin from *Artemisia annua* also gave hope but its use is under threat of resistance. Today quinine and artemisinin, with their synthetic analogues, remain among the most important weapons in our arsenal against malaria. The search for compounds with antimalarial activity continues both from our laboratories and the plants in our forests.

In 2016 about twenty-eight thousand plant species were recorded as being of medicinal use [3], less than 10% of these have been investigated for pharmacological activity. With appropriate development and engagement of materials and funds, there is a resource base to

engage in the war against malaria through investigation of the antimalarial activity extracts and compounds from these plants.

The malaria parasite is fighting back and frustrating therapy through the development of resistance to agents (including ACTs) employed in the conventional treatment of the disease. The spread of resistance is a current concern in the fight against malaria. Efforts to combat malaria will experience great setbacks if the increasing prevalence and severity of artemisinin resistance are not checked.

Phytochemicals are plant-derived chemicals that are useful to plants and humans. Based on their chemical nature they are divided into alkaloids, phenolics (flavonoids and tannins), terpenoids, saponins, and steroids. They are identified and quantified by different methods. Methods of identification may be colorimetric, precipitation, or based on physical properties such as foaming. A quantitative determination may be gravimetric, titrimetric, spectrophotometric, chromatographic, or spectroscopic, among other methods.

The justification of this research is that it will provide more information on the plant and the available potential sources of new antimalarial agents. Although there is research on the plant, the antimalarial evaluation of the stem bark methanol extract has not been carried out (based on our search of available literature).

*Danielliaoliveri*is a traditional medicinal plant used for the treatment of diseases including malaria,in many countries in Africaincluding Nigeria, Kenya, Congo, and Cameroon.

Literature Review

Taxonomical classification

Kingdom	Plantae (Plants)
Sub-kingdom	Viridiplantae (green plants)
Division	Tracheophyta (vascular plants)
Sub-division	Spermatophytina (spermatophyes-seed plants)

Class	Magnoliopsida
Order	Fabales
Family	Caesalpiniaceae (Leguminosae - Caesalpinioideae) (peas,legumes)
Genus	Daniellia Benn
Species	*Danielliaolveri* (Rolfe) Hutch & Dalziel [4]

Taxonomic Serial No.: 506247

Common name(s):

English -African copaiba balsamtree, Ilorin balsam, West African copal, West African gum copal, Santan, African copaiba balsam tree. Arbre à vernis (Fr).; Pau-incenso (Po) [5].

Synonyms: *Paradanielliaoliveri* Rolfe. Trade names: English: African copaiba balsam [6].

"The plant is indigenes to Benin, Burkina, Cameroon, Central African Republic, Chad, Gambia, Ghana, Guinea, Guinea-Bissau, Ivory Coast, Mali, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Togo, Uganda, Zaïre" [7]

Fig 1: Geographical distribution of Danielliaoliveri [7]

Several research investigating various pharmacological activities have been done on the plant. The stem bark methanol extract has been shown to have neuromuscular blocking activity [8]. The oleoresin has antioxidant anti-cytotoxic activities [9, 10]. The antibiotic activity was investigated by [11]. The ethanol extract had antibacterial activity. *S. aureus, B. subtilis, E. faecalis,* and *C. albicans*were more sensitive to ethanol extract than were *E. coli, K. pneumoniae,* and *P. aeruginosa. E.faecalis* was most sensitive at 6.25 mg/ml [12].

"Otherinvestigations include anti-fungal properties- *D. oliveri* bark essential oil showed notable antifungal activity against *Aspergillus niger*and*Trichophytonrubrum* with a minimum inhibitory concentration of 78.1 µg/ml for respectively" [13]; The plant has also shown filaricidal activity-n-hexane extract had (IC50 = 13.9 ± 1.7µg/ml), dichloromethane extract had (IC50 = 22.5 ±0.0µg/ml), and methanol extract had (IC50 = 13.9 ± 1.7µg/ml), against adult *O. ochengi*. n-hexane and dichloromethane extracts were also active against adult *B. pahangi and O. ochengi*mfs [14]. *Danielliaoliveri* demonstrated anti-Alzheimer's disease. Root bark aqueous extract at a dose of 100-300 mg/Kg inhibited the development of neurological abnormalities in amnesic conditions by improving spatial memory formation [14]. An 8 mg/ml) of aqueous extract had Anthelmintic property activity against *Haemonchuscontortus* [15]. Administration of *Danielliaoliveri* leaf ethanol extract at a dose of up to 40 ml/litre to one-day-old broilers (Ross 308) enhanced the overall performance of the birds without any deleterious effect [16]. Feeding one-day-old broiler chicks at 80mg/kg highly influenced the composition of fatty acids and meat quality of animals [17]. The leaves of the plant have demonstrated anti-plasmodial activity [18-20]

Malaria

"Malaria is a vector-borne disease transmitted through the bite of anopheline mosquitoes that are infected with the parasite" [21]. "Malaria is a life-threatening disease caused by the infection of red blood cells with protozoan parasites of the genus. Four species of Plasmodium (*P. falciparum, P. vivax, P. malariae,* and *P. ovale*) most commonly infect humans. *P. falciparum* and *P. vivax* are the most prevalent species and P. falciparum is the most dangerous" [1]. "Malaria transmission intensity is classified into (1): areas of high transmission are characterized by an annual parasite incidence of 450 or more cases per 1000 population and a P. falciparum prevalence rate of 35%" [2]. "Moderate transmission areas have an annual parasite incidence of 250.450 cases per 1000 population and a prevalence of

P. falciparum / P. vivax malaria of 10.35%. (3) Areas of low transmission have an annual parasite incidence of 100-250 cases per 1000 population and a prevalence of P. falciparum/P. vivax of 1-10%. (4) Very low transmission areas have an annual parasite incidence of < 100 cases per 1000 population and a prevalence of *P. falciparum/P. vivax* malaria that is > 0 but < 1%" [1].

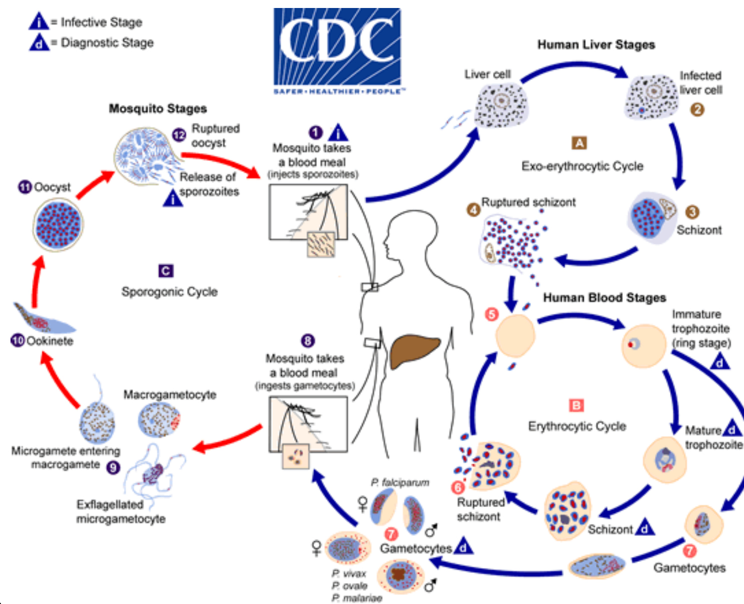

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Fig. 2: Malaria parasite lifecycle [22]

"Microscopic detection of malaria parasites on a thick or thin blood smear is considered the gold standard for the diagnosis of malaria. Other methods include Rapid diagnostic tests (RDT), or polymerase chain reaction (PCR)" [2]. "Several strategies have been used to prevent malaria. They include (1) Mass drug administration (MDA), (2) Perennial malaria chemoprevention (PMC) (3) seasonal malaria chemoprevention (SMC) (4) targeted drug administration (TDA), (5) reactive drug administration (RDA)" [1]. "Other approaches include the use of Insecticide-treated nets INTs), indoor residual spraying (IRS) campaigns, and seasonal malaria chemoprevention SMC" [23]. Intermittent Preventive Therapy (IPT) [21], and the administration of malaria vaccine: [1,2].

“The World Health Organization (WHO) recommends artemisinin-based combination therapies (ACTs) for the treatment of malaria. The artemisinin component rapidly clears parasites from the blood (reducing parasite numbers while the longer-acting partner drug clears the remaining parasites and provides protection against the development of resistance to the artemisinin derivative. Partner drugs with longer elimination half-lives also provide a period of post-treatment prophylaxis” [2]. In the absence of resistance to the partner drug, the five recommended ACTs have all been shown to achieve a PCR- an adjusted treatment failure rate of 5% in many trials in several settings in both adults and children. WHO-approved first-line ACT options are:

artemether + lumefantrine

artesunate + amodiaquine

artesunate + mefloquine

dihydroartemisinin + piperaquine

artesunate + sulfadoxine-pyrimethamine (SP)

artesunate + pyronaridine

“For the treatment of severe malaria (including in pregnant women) full doses of effective parenteral (or rectal) antimalarial treatment must be given promptly in the initial treatment of severe malaria. Two classes of medications are available for the parenteral treatment of severe malaria: artemisinin derivatives (artesunate or artemether) and cinchona alkaloids (quinine and quinidine). Parenteral artesunate is the treatment of choice for all severe malaria” [2]. “Parenteralantimalarial drugs should be given to pregnant women with severe malaria in full doses without delay. Parenteral artesunate is the treatment of choice in all trimesters. Treatment must not be delayed. If artesunate is unavailable, intramuscular artemether should be given, and if this is unavailable then parenteral quinine should be startedimmediately until artesunate is obtained” [1].

Materials and Methods

Collection and preparation of plant materials

The stem bark of *Danielliaoliveri*was collected in the forests of Iha-alumuna in Enugu state of Nigeria. Itwas identified by the herbarium of the Faculty of Pharmaceutical Science, University of Nigeria, Nsukka. A sample with voucher number PCG/UNN/0374 was deposited there. The fresh stem barks were cut into pieces, dried under shade, and pulverized using a mechanical blender. It was sequentially extracted with n-hexane, then, followed by methanol. The extracts were concentrated with a rotary evaporator and placed in a refrigerator till further use.

Equipment

Shimadzu ATX224 Analytical Balance, Agilent InfinityLab LC/MSD equipment, Model number - G6125B, Model UV 7 UV/Visible Spectrophotometer, Manual single channel micropipette (Pipet-Lite XL Model, Mettler-Toledo Inc., Columbus, USA), test tubes, beakers, spatula, glass stirring rods, Whatman No 1 filter paper.

Reagents

Dragendorff's reagent, picric acid, Fehling's solutions A and B, rutin, Methanol, conc. Sulphuric acid concentrated ammonia (Merck KGa A, Darmstadt, Germany), gallic acid, potassium ferricyanide, NaOH, acetic anhydride, (Reagents, Charlotte, NC 28214, USA) ferric chloride (Xilong Scientific Co., Ltd, China) Na2CO3, ZoupingZhijin New Material Technology Co., Ltd Shandong, China), Cholesterol Fissions Chemicals (United Kingdom) atropine and vanillin (Sigma Chemical, USA), diosgeninXiangyang Wellbeing Pharmchem Co., China), linalool (Basf Se, Belgium), Giemsa stain, Artemether, Lumefantrine

Malarial parasite: *Plasmodium berghei* (ANKA)

Phytochemical analyses Methodology

Qualitative and quantitative Phytochemical analyses of samples were performed according to the methods of [24-26].

Quantitative phytochemical analysis

1 Determination of alkaloid

A 1g of the extract was macerated with 20% H_2SO_4 for 3hrs and filtered. A 1ml of the filtrate was mixed with 5ml of 60% H_2SO_4, mixed well, and allowed to stand for 3 hours. The absorbance was read at 490nm. All determinations were performed in triplicate and the result was expressed as the Mean ±Standard deviation. Total alkaloid content was as calculated from a regression equation for an atropine standard curve.

2 Estimation of Total Saponin determination

Vanillin–Sulfuric acid assay as described by Anh et al., (2018), slightly modified was used to determine the saponin content of the extract. 0.5 ml of the sample solution, 0.5 ml of vanillin solution of 8% (w/v), and lastly 5.0 ml of sulfuric acid of 72% (w/v) were added and mixed in an ice water bath. After the mixture was then warmed in a bath at 60 ˚C for 10 minutes and then cooled in ice–cold water, the absorbance was measured at 527 nm. All determinations were performed in triplicate and the result was expressed as the Mean ±Standard deviation. Saponin concentration was calculated from a standard saponin calibration curve of the standard. The saponin content was expressed as mg/100g saponin equivalents from the regression equation

3 Determination of Tannin Content

Tannin content was determined by Van-Burden and Robinson (1981) method with slight modifications. A 1 ml of the extract solution (0.1g/10ml) in de-ionized water was pipetted out into the test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.1 N HCl and 1 ml of 0.008M

K3Fe(CN)6 (potassium ferricyanide). The absorbance was measured at 530 nm within 10 minutes. Tannic acid was used as a reference standard and all determinations were performed in triplicate. The tannin content was expressed as mg of tannic acid equivalents (TA)/100g of extract using the regression equation for the standard

4 Estimation of Steroids

1ml of the solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water bath maintained at 70±20C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank. The calibration curve for the cholesterol standard was preparedand steroid content was calculated from the regression equation. The steroid content was expressed as mg/100g cholesterol.

6 Determination of Flavonoid Content

Total flavonoid content was estimated by the aluminum chloride colorimetric method.A 0.1m of the extract was placed in a test tube together with distilled water (3.9ml). Thenwas added 0.3 ml of % $NaNO_2$, after 5 minutes 0.3ml of 10% $AlCl_3$ and after another 5 minutes 2ml of 1 M NaOH followed by the addition of distilled water to make up to 10ml. The absorbance was measured against the blank at 510 nm after 15 minutes. All determinations were performed in triplicate and the result was expressed as the Mean ±Standard deviation. The standard curve was prepared using different concentrations of rutin. The flavonoid content was expressed as mg/g rutin equivalents from the regression equation

7 Estimation of Total Phenolics

The folin-Ciocalteu method was used for the analysis of the total phenol of the plant extracts. To 0.1 ml of the extract, 3.9ml of distilled water, and 0.5ml of Folin's reagent were added. The tube was incubated at room temperature for 10 minutes. To this was added 2ml of 25%

sodium carbonated andthe test tube was kept in a boiling water bath for 1 minute. The absorbance was read at 650nm. All determinations were performed in triplicate and the result was expressed as the Mean ±Standard deviation. The total phenolic concentration was read from a gallic acid standard curve. Thetotal phenolics content was expressed as mg/100 g gallic acid equivalents (GAE) from the regression equation

8 Estimation of cyanogenic glycosides:

1.0ml of the filtrate was pipette into a test tube, and 4ml of alkaline picrate solution was added and incubated for 5 minutes in a water bath at 90℃. The test tube was cooled to room temperature and the absorbance of the solution was recorded at 490nm. All determinations were performed in triplicate and the result was expressed as the Mean ±Standard deviation. The concentration of cyanide in the sample was determined from a regression equation of the standard

HPLC-MS

HPLC-MS was also conducted using Agilent InfinityLab LC/MSD Model number - G6125B Serial number - SG1932N001 with Eclipse plus C18 5.0µm 4.6mm x 250 mm column and data was processed with Agilent OpenlabChemstation software.

Protocol for LCMS Analysis

The samples were analyzed using liquid chromatography (LC) mass spectrometer (MS). The extracted samples were reconstituted in Methanol and filtered through a polytetrafluoroethylene (PTFE) membrane filter of 0.45 µmsize. After filtration, the filtrate (20.0 µl) was injected into the LC system and allowed to separate.The run was carried out at a flow rate of 1.0 mL/min, with Sample and Column temperature at 40°C. The mobile phase consists of 0.1% formic acid in water (solvent A) and 0.1% acetic acid in Methanol (solvent B) with a gradient as below

Table 1: HPLC gradient elution

Time(min)	%A	%B
0	70	30
10	50	50
14	50	50
21	10	90
25	10	90
25.10	70	30
32	70	30

The DA detector was set at two wavelengths 220& 231nm with a resolution of 1.2nm and a sampling rate of 10 points/sec. The mass spectra were acquired with a scan range from m/z 100 – 1000 after ensuring the following settings: ESI source in positive and negative ion modes; capillary voltage 3.5kv (positive) and 3.0kv (negative); probe temperature 350°C; flow rate 10 mL/min; nebulizer gas, 50 psi. The data was processed with Agilent OpenlabChemstation software. The compounds were identified based on the following information, elution order, retention time (tR), fragmentation pattern, and base m/z.

Acute toxicity test

An acute toxicity test was done according to OECD 423 standard guidelines [28]

Antimalaria activity screening

Percent parasitemia and survival time were used to assess the therapeutic potency of the extract.

In vivo antimalarial efficacy was examined by evaluating percent suppression, percentage inhibition percent survival, and mean survival time.

Animals

Inbred male and female (non-pregnant) Swiss albino mice (18–25 g) were obtained from the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The animals were maintained under conventional conditions of 12 h light/ dark cycle in standard cages. They were freely fed with a pellet diet and water. After an acclimatization period of 7 days, mice were randomly divided into six experimental groups.

Curative Anti-malarial activity screening

Preparation

A combination of (artemether + lumefantrine) was used as a standard antimalarial drug in this study. The drug at chosen dose was freshly prepared in 2% tween 80 and administered orally by gavage. The drug dose, (artemether + lumefantrine) (1.14/6.86 mg/kg/d).

was adjusted at the time of administration according to the weight of the mice. dose of

A muse was infected with *Plasmodium berghei* (ANKA). When the parasitemia in the donor mouse was 20%, infected mouse blood was then collected by cardiac puncture and suspended in phosphate bu□er saline (PBS). The blood was then diluted with physiological saline (0.9%) of normal mice in such a way that 1 ml of blood contains 5×10^7 infected RBCs.

Each mouse was then given 0.2 ml of this diluted blood intra-peritoneally which contained

1×10^7*P. berghei* infected. on the 1st day of the experiment (Day 0).

Parasite density was determined daily for five days, by counting the number of parasitized erythrocytes in 10 different fields. Parasitemia was estimated by microscopic observation of completely dried thin slide films prepared from each assay well under the x100 objective in oil immersion using the Olympus binocular microscope, (Model CH30 Japan) (WHO 1991; NMIMR/SOP). A minimum of 1000 RBCs were counted. The survival time for each group was determined by finding the average survival time (days) of mice, starting from their infection in each group for 30 days (D0 - D29) [29]

Experimental:

Group 1 Toxic group (parasitized received neither the extract nor the standard drug)

Group 2 Normal group (the negative control non-parasitized, given feed and water)

Group 3 Standard group (parasitized and received [Artesunate+ Lumefantrine])

Group 4 200mg of extract/Kg b. wt. (parasitized and received the extract)

Group 5 400mg extract/Kg b. wt. (parasitized and received the extract)

Group 6 600mg extract/Kg b. wt. (parasitized and received the extract)

Estimate of % parasitemia was determined by the relation:

% parasitemia = [(no. of infected cells ÷ total cell count) × 100%]

The % parasitemia was determined for a particular extract concentration, this was transformed into percentage inhibition of growth by comparison with control values.This is given by the relation: [(a – b)/a] x 100%

Where a=% parasitemia in (untreated) control and b= % parasitemia in extract (standard drug) treatment.

Data and statistical analysis

The average parasitemia was determined by obtaining the percentage of the ratio of parasitized to the total number of RBCs. The average percentage chemo-suppression (or parasite clearance) was calculated as 100 × [(A–B)/A], where A is the average parasitemia of the negative control group and B is the average parasitemia of the test group.

One-way analysis of variance between groups (ANOVA) and post hoc Duncan and Tukey Post hoc tests were used to compare data for the treatment groups at $p = 0.5$

Mortality in the mice was followed up to 28 days post-infection to evaluate the percent survival and mean survival time.

Animals were checked for these symptoms twice a day until the end of the experiment. The animals were humanely euthanized with chloroform.

Results

Antimalarial activity:

Danielliaoliveri bark methanol extract has "High" anti-plasmodial activity, 81.1% reduction in parasitemia at a dose of 600mg/kg body weight. A dose-dependent pattern was observed. Mean survival time was excess 5. The 600 mg/Kg body weight had 96 % of the activity of the standard.

Table 2: Antimalarial result

Group	% suppression
Grup 3 Std (Art + Lumet)	84.81
Grup 4 (100mgof extract)	26.97
Grup 5 (400mgof extract)	60.64
Grup 6 (600mgof extract)	81.11

Table 3: ANOVA
Parasite level

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3047941332.160	4	761985333.040	2498.636	.000
Within Groups	6099210.400	20	304960.520		
Total	3054040542.560	24			

Table 4: Parasite level Post hoc

			Subset for alpha = 0.05				
	Group	N	1	2	3	4	5
Tukey HSD[a]	Standard (Std (Art + Lumet) Grp	5	4902.20				
	Extract (600 mg/Kg B. wt.	5		6469.80			

		N	1	2	3	4	5
	Extract (40 0mg/Kg B. wt.	5			12869.80		
	Extract (10 0mg/Kg B. wt.	5				24673.80	
	Untreated (Toxic) Grp	5					33660.60
	Sig.		1.00	1.00	1.00	1.00	1.00
Duncan[a]	Standard (Std (Art + Lumet) Grp	5	4902.20				
	Extract (600 mg/Kg B. wt.	5		6469.80			
	Extract (40 0mg/Kg B. wt.	5			12869.80		
	Extract (10 0mg/Kg B. wt.	5				24673.80	
	Untreated (Toxic) Grp	5					33660.60
	Sig.		1.000	1.00	1.000	1.00	1.00

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

Table 5; Hematological Indices analysis

	%PCV	**RBC x 10^{12}**	**HB g/dl**	**WBC $10^6/mm^3$**	**PLATELET x 10^9/L**	**% N**	**% L**	**% M**	**%**
Un-treated Group	30.00±1.34[a]	7.40±.2[a]	9.00±.0[a]	4.1[a]	88.80±.74 [a]	61.00±.45	31.00±.00	5.40±.25 a	1 a

Normal group	37.00±.7^{b}	9.44±.2^{b}	11.98±.2^{b}	5.44 ^{a}	103.20±3.07	64.00±6.03	37.80±.86 ^{b}	3.20±.37 ^{b}	1 ^{b}
Standard group	36.00±.9^{b}	9.44±.1^{b}	11.68±.2 ^{b}	4.48 ^{a}	95.20±2.52	57.80±1.11 ^{a}	38.40±.81 ^{b}	3.20±.37 ^{b}	1 ^{b}
200mg/Kg group	35.00±2.2^{b}	8.14±.2^{b}	10.82±.7 ^{b}	4.20 ^{a}	82.00±.89	52.00±.89 ^{a}	43.60±.68	3.40±.25 ^{b}	1 ^{b}
400mh/Kg group	32.40±.2^{c}	9.54±.0^{c}	10.90±.1 ^{b}	4.10 ^{b}	89.00±.445 ^{a}	54.00±.89 ^{a}	41.20±.92	2.60±.25 ^{b}	2 ^{a}
600mh/Kg group	35.50±2.2^{b}	9.04±.1^{b}	11.48±.2 ^{b}	5.18 ^{a}	93.20±2.52	59.00±.89 ^{a}	37.40±.81 ^{b}	3.15±.37 ^{b}	1 ^{b}

N= 5; Data expressed as Mean ±SEM; Data in the same column with the same superscript are not significantly different

Table 6: Quantitative Phytochemical Analysis

Parameter	**Quantity (mg/100g)**
Alkaloids	10.179±0.61
Saponins	1.674±0.43
Tannins	10.738±0.61
Flavonoids	3.923±0.15
Steriods	2.665±0.07
Cyanogenic glycosides	NIL
Phenols	134.604±14.83
Terpernoids	22.436±4.87

The HPLC-MS analysis revealed 56 compounds while the HPLC chromatogram showed 9 prominent peaks.

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Discussion

Malaria caused an estimated 241 million cases and 627,000 deaths globally in 2020 [1]. ACTs have activity against both asexual blood-stage and sexual stages of the malaria parasite but do not clear parasites in the liver. They demonstrate greater than 95% clinical efficiency in PCR test. Artemisinin resistance is characterized by a reduced rate of parasite clearance and creates the opportunity for the development of resistance. The spread of resistance is a current concern in the fight against malaria. Efforts to end malaria is will experience great setbacks if the increasing prevalence and severity of artemisinin resistance are not checked. This has created an urgent need to develop new antimalarial agents to which there is no resistance [30].

Results of the two endpoints in antimalarial assay % parasitemia suppression (>90%), and mean survival time (excess 5), are impressive and encouraging, indicating the potential of the plant as a source of novel antimalarial agents are impressive. An ACT (artemether + lumefantrine) was used as a standard based on the current WHO recommendation for the treatment of malaria. Our choice of - *Plasmodium berghei* as a rodent infecting organism was based on literature research.

"Prediction of the hematological changes in malaria enables early therapeutic interventions to prevent the occurrence of major complications. Therefore, malaria patients should be checked for the presence of hematological abnormalities such as anemia, and have to be managed for these abnormalities. Presumptive treatment is enabled by hematological parameters when a parasitological examination is not immediately available or uncertain" [31], and "helps to prevent death arising from such complications" [32]. "In this research, the mean values of % PCV, RBC, HB, lymphocyte level, and WBC, were significantly lower in the untreated group compared to the standard drug and extract treated groupswhile thrombocyte and neutrophil counts were elevated. This is in agreement with the work of" [33].

Group 1 (the group uninfected group 0%) andgroup 2 the toxic (reference-100 % parasitemia) are not represented because of their level of parasitemia, 0 and 100%. In this research hematological parameters showed a significant difference between the extract-treated groups and the untreated group.

In addition to the fact that some compounds could have co-eluted in the liquid chromatographic separation, some compounds may not appear in the HPLC result but will be captured by the MS analysis. A previous work, [9], on the chemical composition of the oleoresin from Danielliaoliveri (Rolfe) Hutch. &Dalz. (Caesalpiniaceae) using GC-FID/GC-MS identified mostly volatile diterpenoids. This is a possible explanation for the discrepancy in the number of peaks in HPLC and MS.

Many bioactive compounds with potential antimalarial activities have been identified in plants. The plant is a potential source of many drug candidates considering the concentration of phenolics, terpenoids, and alkaloids as reflected by the quantitative phytochemical analysis result, and the broad spectrum of pharmacological activities of these classes of phytochemicals.

Conclusion

Danielliaoliveri stem bark methanol extract contains many secondary metabolites and has "high"anti-*Plasmodium berghei* activity, 81.1% reduction in parasitemia at a dose of 600mg/kg body weight.*Danielliaoliveri* is a good antimalarial medicinal plant and could be a source of antimalarial compounds.

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Competing Interests:

None to declare

Ethical approval:

Ethical approval was obtained from the Research Ethics Committee of the University of Nigeria, Nsukka.

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