

Original Research Article

Antimalarial Assay, Isolation and Characterization of Compound Responsible for Antimalarial Activity in *Daniellia oliveri* (Rolfe) Hutch. & Dalziel (Caesalpinaceae).

Abstract

Malaria is still a global health problem though a lot of resources have been channeled into its prevention and elimination. The bark dried, powdered bark of *Daniellia oliveri* was extracted with n-hexane followed by methanol. Methanol extract of *Daniellia oliveri* was tested for antimalarial (curative test) by being tested against *Plasmodium berghei* in mice with doses 100, 400 and 600 mg/kg. Acute toxicity studies were carried out according to OECD 423 protocol, and phytochemical screening using standard protocols. HPLC-MS was also conducted using Agilent InfinityLab LC/MSD with Eclipse plus C18 5.0 μ m 4.6mm x 250 mm column, and data was processed with Agilent Openlab Chemstation software. Preliminary phytochemical screening of the extract revealed the presence of flavonoids, tannins, alkaloids, phenolics, and terpenoids. The methanol extract was found to be safe at doses up to 5000 mg/kg body weight (acute lethal dose, LD₅₀) in mice. One-way analysis of variance between groups (ANOVA) and Duncan and Tukey Post hoc tests were used to compare data for the treatment groups. There was a significant difference between the groups at (p<0.05). The HPLC-MS analysis showed the presence of 56 compounds. *Daniellia oliveri* bark methanol extract has “High” anti-plasmodial activity, an 81.1% reduction in parasitemia at a dose 600mg/kg body weight and Mean survival time of excess 5. *Daniellia oliveri* is a potentially “good” human antimalarial medicinal plant.

Key words: *Daniellia oliveri*; Malaria; *Plasmodium berghei*; ACT; HPLC-MS; phytochemistry

Introduction

Globally, malaria deaths reduced steadily over the period 2000–2019, from 896 000 in 2000 to 562 000 in 2015 and to 558 000 in 2019. In 2020, malaria caused an estimated 627 000; an estimated deaths [1]. According to the latest report, there were an estimated 241 million cases and 627 000 deaths globally in 2020. 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 [2]. Malaria is a common cause of febrile illness in areas where it is transmitted; therefore, the diagnosis and management of malaria should routinely be considered for any febrile person who has traveled to an area with malaria in the weeks to months preceding symptom onset [3].

Plants with known medicinal uses have been a source of vital pharmaceutical drugs for the treatment of many diseases. For example, artemisinin (discovered in *Artemisia annua*) and quinine (from *Cinchona officinalis*), together with their synthetic analogues, remain among the most important weapons in our arsenal against malaria. 28,187 plant species are currently recorded as being of medicinal use [4].

Literature Review

The plant *Daniellia oliveri* (Rolfe) Hutch. & Dalziel (Caesalpinaceae)

The plant

Daniellia oliveri (Rolfe) Hutch, & Dalziel

Taxonomy

Kingdom Plantae (Plants)

Sub-kingdom Viridiplantae (green plants)
 Division Tracheophyta (vascular plants)
 Sub-division Spermatophytina (spermatophytes-seed plants)
 Class Magnoliopsida
 Order Fabales
 Family Caesalpiniaceae (Leguminosae - Caesalpinioideae) (peas, legumes)
 Genus *Daniellia* Benn
 Species *Daniellia olveri* (Rolfe) Hutch & Dalziel [5]

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). [6]
 Synonyms: *Paradaniellia oliveri* Rolfe

Trade names: English: African copaiba balsam [7]

The plant is indigenous to Benin, Burkina, Cameroon, Central African Repu, Chad, Gambia, Ghana, Guinea, Guinea-Bissau, Ivory Coast, Mali, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Togo, Uganda, Zaire [8]

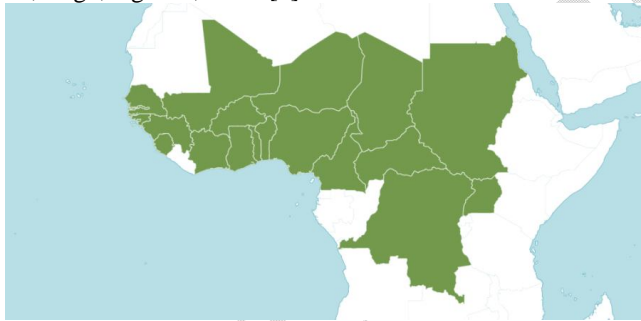


Fig 1: Geographical distribution of *Daniellia oliveri*

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Description

Daniellia oliveri is a medium-sized, deciduous tree growing to a height of 25 m (80 ft) or more. It has a sometimes twisted trunk up to 200 cm (80 in) in diameter, and a broad, flat-topped crown, and usually lacks branches on the lowest 9 m (30 ft) of trunk [9]. The bark is greyish-white, smooth at first but later flaking off in patches. The alternate leaves are pinnate, up to 15 cm (6 in) long, with six to eleven pairs of leaflets and no terminal leaflet. The inflorescence is a compound raceme, the individual scented bisexual flowers having five, unequal creamy-white petals. These are followed by flattened oblong pods each containing one seed [10]. Inflorescence an axillary or terminal compound raceme 15–20(–25) cm long, glabrous to densely hairy, with 6–16 lateral branches. Flowers bisexual, zygomorphic, scented; pedicel 7–13 mm long, glabrous, enlarging in fruit, below the middle with 2 caducous bracteoles c. 0.5 cm long; Fruit an obliquely lanceolate, flattened pod 6–10 cm × 3–4.5 cm, with stipe about 1 cm long, glabrous, green becoming brown, dehiscent with 2 papery valves, 1-seeded. Seeds obovoid-ellipsoid, flattened, 2–2.5 cm long, smooth, dark brown, attached to one of the valves by a 1–2 cm long funicle [11]. Morphology: *Daniellia oliveri* is a slow-growing, deciduous tree with a flat-topped, spreading, dense crown; usually growing 9–25 metres tall, but with occasional specimens as tall as 45 metres. The cylindrical bole,

which can be straight or twisted, can be 150 - 200cm in diameter, unbranched for the first 8 - 10 metres; unbuttressed but with root flutes at the base[12].

Uses

Useful Part(s): Gum, bark, Leave, Gum

General Uses: Food and Medicinal

Medicinal Uses

A decoction of the root is used in the treatment of gonorrhoea and skin diseases. The gum-resin, obtained from the wood, is used medicinally. The leaves are used in the treatment of dysmenorrhea [617]. The roots, leaves and the bark are used medicinally [13].

Aqueous extract of the bark is used for treatment of pain, an infusion of the bark is used to relief headache, A decoction of the root is used to treat gonorrhea and skin diseases [14].

Ethnomedicinal uses

Daniellia oliveri (DO) is a traditional medicinal plant used for the treatment of diseases such as inflammation, schizophrenia, and epilepsy in Nigeria, Kenya, Congo, and Cameroon [15].

Pharmacological Activities

A number of researches investigating various pharmacological activities have been done on the plant. The stem bark methanol extract has been shown to have neuromuscular blocking activity[16] The oleo resin has antioxidant and cytotoxic activities [17]. The antibiotic activity was investigated by [18], Anti-fungal properties [19], Filaricidal activity [20] Antiplasmodial activity [21-23]

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Malaria

Malaria is a vector-borne disease transmitted through the bite of anopheline mosquitoes that are infected with the parasite [24]. 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 [25]. 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% [26] 26 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% [2].

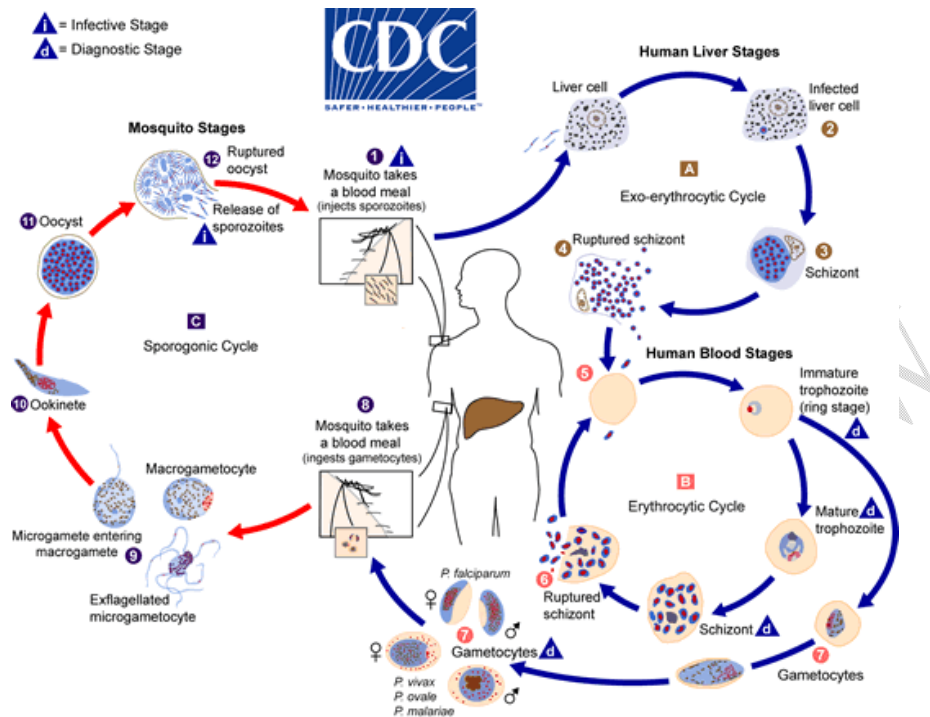


Fig. 2: Malaria parasite lifecycle [25]

Diagnosis

Prompt, accurate diagnosis of malaria is an important part of effective case management. A diagnosis of malaria requires confirmation through either microscopy, RDT, or polymerase chain reaction (PCR). The microscopic detection of the malaria parasite on a thick or thin blood smear is considered the gold standard for diagnosis, but reliable, high-quality microscopy is rarely available. In such situations, RDTs to detect specific malaria antigens in a capillary blood sample are routinely used, including at most peripheral health facilities. Diagnosis using PCR to detect parasites in blood samples or dried blood spots is primarily used in research settings [2].

Prevention

A number of strategies have been used to prevent malaria. They include (1) Mass drug administration (MDA), the administration of a full therapeutic course of an antimalarial medicine at approximately the same time, and often at repeated intervals, to all age groups of a population in a defined geographical area. Antimalarial medicines are administered without prior malaria testing and therefore regardless of the malaria infection status of individuals. (2) Perennial malaria chemoprevention (PMC) (3) seasonal malaria chemoprevention (SMC) (4) targeted drug administration (TDA), (5) reactive drug administration (RDA). All of these strategies share a common underlying principle – that the provision of a treatment dose of antimalarial medicine will cure existing infections and prevent new ones [2]. Other approaches include use of Insecticide-treated nets (ITNs), indoor residual spraying (IRS)

campaigns, seasonal malaria chemoprevention SMC. SMC is the intermittent administration of full treatment courses of an antimalarial medicine (currently amodiaquine plus sulfadoxine-pyrimethamine) to children during the malaria season in settings with highly seasonal malaria transmission patterns [26].

Intermittent Preventive Therapy

Intermittent preventive treatment of malaria in infants (IPTi) (IPTi) and (IPTp). Intermittent preventive treatment of malaria in pregnancy. Intermittent preventive treatment of malaria in infants (IPTi) is the administration of a full therapeutic course of an antimalarial drug, sulfadoxine-pyrimethamine, to infants during 3 of their routine visits for immunization in the first year of life. To prevent malaria in pregnancy, WHO recommends intermittent preventive treatment in pregnancy (IPTp) with one dose of sulfadoxine-pyrimethamine offered at each scheduled antenatal care visit (maximum monthly) after the first trimester [27,28].

Malaria vaccine: The World Health Organization (WHO) recommended RTS,S/AS01 (RTS,S), the world's first malaria vaccine, for children at risk in sub-Saharan Africa and in other regions with moderate to high transmission of malaria caused by *Plasmodium falciparum* in 2021 [29]. The RTS,S/AS01 malaria vaccine should be used for the prevention of *P. falciparum* malaria in children living in regions with moderate to high transmission as defined by WHO [2].

Treating uncomplicated malaria

Definition of uncomplicated malaria

A patient who presents with symptoms of malaria and a positive parasitological test (microscopy or RDT) but with no features of severe malaria is defined as having uncomplicated [2]. The World Health Organization (WHO) recommends artemisinin-based combination therapies (ACTs) for the treatment of malaria. Artemisinin and its derivatives are potent and fast-acting drugs that cause a rapid decline in parasitemia during the first days of treatment [30]. ACT is a combination of a rapidly acting artemisinin derivative with a longer-acting (more slowly eliminated) partner drug. The artemisinin component rapidly clears parasites from the blood (reducing parasite numbers by a factor of approximately 10 000 in each 48 hours asexual cycle) and is also active against the sexual stages of the gametocytes that mediate onward transmission to mosquitoes. The longer-acting partner drug clears the remaining parasites and provides protection against 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-adjusted treatment failure rate of 5% in many trials in several settings in both adults and children.

The WHO-approved first-line ACT options are:

- ✓ artemether + lumefantrine
- ✓ artesunate + amodiaquine
- ✓ artesunate + mefloquine
- ✓ dihydroartemisinin + piperaquine
- ✓ artesunate + sulfadoxine-pyrimethamine (SP)
- ✓ artesunate + pyronaridine

Artesunate pyronaridine is included in the WHO list of prequalified medicines for malaria, the Model List of Essential Medicines and the Model List of Medicines for Children. The drug has also received a positive scientific opinion from the European Medicines Agency and

undergone a positive review by the WHO Advisory Committee on Safety of Medicinal Products [2].

Treatment of severe malaria

It is essential that full doses of effective parenteral (or rectal) antimalarial treatment be given promptly in the initial treatment of severe malaria. This should be followed by a full dose of effective ACT orally. Two classes of medicine are available for parenteral treatment of severe malaria: artemisinin derivatives (artesunate or artemether) and the cinchona alkaloids (quinine and quinidine). Parenteral artesunate is the treatment of choice for all severe malaria [2].

Treatment of severe malaria during pregnancy

Parenteral antimalarial 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 started immediately until artesunate is obtained [2].

Artemisinin-resistance

Artemisinin resistance¹ is defined as delayed parasite clearance following treatment with an artesunate monotherapy or with an artemisinin-based combination therapy (ACT). This represents partial resistance [30].

There is concern that resistance is spreading. An increased prevalence, severity, or both of artemisinin resistance would be an enormous setback in the efforts to end malaria. With this in mind, novel approaches to accelerate the development efforts of new malaria therapeutics are urgently needed [30].

Phytochemistry

Phytochemicals are plant-derived chemicals which are useful primarily to the plants, then to humans. When the chemical are of little importance to the plants but are immensely useful to human they are referred to as secondary metabolites. Secondary metabolites are produced as waste products from metabolic processes or in response to stress factors. Phytochemicals can be classified based on different considerations including their chemical composition, pharmacological activities, etc. Based on their chemical nature they are divided into alkaloids, phenolics (flavonoids and tannins), terpenoids, saponins, steroids. They are identified and quantified by different methods. Methods of identification may be colorimetric, precipitation, or based on physical properties such as foaming. Quantitative determination may be gravimetric, titrimetric, spectrophotometric, chromatographic, or spectroscopic, among other methods. At times two methods could be combined for greater efficiency, e.g. GC-MS, LC-MS techniques.

Materials and Methods

Collection and preparation of plant materials

The plant material, stem bark of *Daniellia oliveri* was collected in the forests of Iha-alumuna in Enugu state of Nigeria. It was 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 extracted 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, Whatmann 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, Zouping Zhijin New Material Technology Co., Ltd Shandong, China), Cholesterol Fissions Chemicals (United Kingdom) atropine and vanillin (Sigma Chemical, USA), diosgenin Xiangyang Wellbeing Pharmchem Co., China), linalool (BASF Se, Belgium), Giemsa stain

Malarial parasite: *Plasmodium berghei* (ANKA)

Phytochemical analyses Methodology

Qualitative and quantitative Phytochemical analyses of samples were performed according to the methods of (Evans, 2009; Harborne, 1973; Harborne, 1998; Sofowora, 2008).

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 Openlab Chemstation 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 polytetrafluoroethylene (PTFE) membrane filter with 0.45 µm size. 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, 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 resolution of 1.2nm and sampling rate at 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 Openlab Chemstation software. The compounds were identified on the basis of the following information, elution order, and retention time (tR), fragmentation pattern, and Base m/z.

Acute toxicity test

Acute toxicity test was done according to OECD 425 standard guidelines [31]

Antimalaria activity screening

Percent parasitemia and survival time was 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 pellet diet and water. After acclimatization period of 7 days, mice were randomly divided into six experimental groups.

Curative Anti-malarial activity screening

Preparation

A combination of Artesunate+ Lumefantrine was used as standard antimalarial drug in this study. The drug at chosen dose was freshly prepared in 2% tween 80 and administered orally by gavage. Drug dose, expressed in mg/kg of body weight, was adjusted at the time of administration according to the weight of the mice. dose of artemeter + lumefantrine 1.14/6.86 mg/kg/d).

A mice 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 buffered saline (PBS). The blood was then diluted with physiological saline (0.9%) of normal mice in such a way that 1 ml 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 at least 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 against

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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)[32]

Group 1	Toxic group. (parasitized received neither the extract nor the standard drug)
Group 2	Normal group (the negative control non-parasitized blood, given feed and water)
Group 3	Standard group (parasitized and received the standard drug artemeter + lumefantrine)
Group 4	200mg of extract/Kg bw (parasitized and received the extract)
Group 5	400mg extract/Kg (parasitized and received the extract)
Group 6	600mg extract/Kg (parasitized and received the extract)

Estimate of % parasitemia was determined by the relation:

$$\% \text{ parasitemia} = [(\text{no. of infected cells} \div \text{total cell count}) \times 100\%]$$

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: $[(a - b)/a] \times 100\%$

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 RBC. Average percentage chemo-suppression (or parasite clearance) was calculated as $100 \times [(A-B)/A]$, 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

Mortality in the mice was followed up to 28 day 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:

Daniella oliveri bark methanol extract has "High" anti-plasmodial activity, 81.1% reduction in parasitemia at a dose 600mg/kg body weight A dose dependent pattern was observed.

Mean survival time was excess 5. The 600 mg/Kg B. wt. had 96 % the activity of the standard

Table 2: Antimalarial result

Group	% Suppression
Grup 3 Std (Art + Lumet)	84.81
Grup 4 100mg	26.97

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Grup 5 400mg	60.64
Grup 6 600mg	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

	Group	N	Subset for alpha = 0.05				
			1	2	3	4	5
Tukey HSD ^a	Standard (Std (Art + Lumet) Grp	5	4902.200				
	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.		1.00	1.00	1.00	1.00	1.00
Duncan ^a	Standard (Std (Art + Lumet) Grp	5	4902.200				
	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	%E
Un-treated Group	30.00±1.34 ^a	7.40±.2 ^a	9.00±.0 ^a	4.1x10 ^{3a}	88.80±.74 ^a	61.00±.45	31.00±.00	5.40±.25 ^a	1.84±.45 ^a
Normal	37.00±.7 ^b	9.44±.2 ^b	11.98±.2 ^b	5.44	103.20±3.07	64.00±6.03	37.80±.86 ^b	3.20±.37 ^b	1.80±.20 ^p

group				$\times 10^3$ ^a					
Standard group	36.00 \pm .9 ^b	9.44 \pm .1 ^b	11.68 \pm .2 _b	4.48 $\times 10^3$ ^a	95.20 \pm 2.52	57.80 \pm 1.11 _a	38.40 \pm .81 _b	3.20 \pm .37 _b	1.60 \pm .25 _b
200mg/Kg group	35.00 \pm 2.2 ^b	8.14 \pm .2 ^b	10.82 \pm .7 _b	4.20 $\times 10^3$ ^a	82.00 \pm .89	52.00 \pm .89 ^a	43.60 \pm .68	3.40 \pm .25 _b	1.00 \pm .00 _b
400mg/Kg group	32.40 \pm .2 ^c	9.54 \pm .0 ^c	10.90 \pm .1 _b	4.10 $\times 10^3$ ^b	89.00 \pm .445 ^a	54.00 \pm .89 ^a	41.20 \pm .92	2.60 \pm .25 _b	2.60 \pm .25 _a
600mg/Kg group	35.50 \pm 2.2 ^b	9.04 \pm .1 ^b	11.48 \pm .2 _b	5.18 $\times 10^3$ ^a	93.20 \pm 2.52	59.00 \pm .89 ^a	37.40 \pm .81 _b	3.15 \pm .37 _b	1.80 \pm .20 _b

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

Phytochemical analysis:

The qualitative Phytochemical analysis revealed the presence of Alkaloids, Flavonoids, Tannins, Cardiac glycosides, Reducing sugar, Saponins, Terpenoids, Phenols.

Table6: Quantitative Phytochemical Analysis

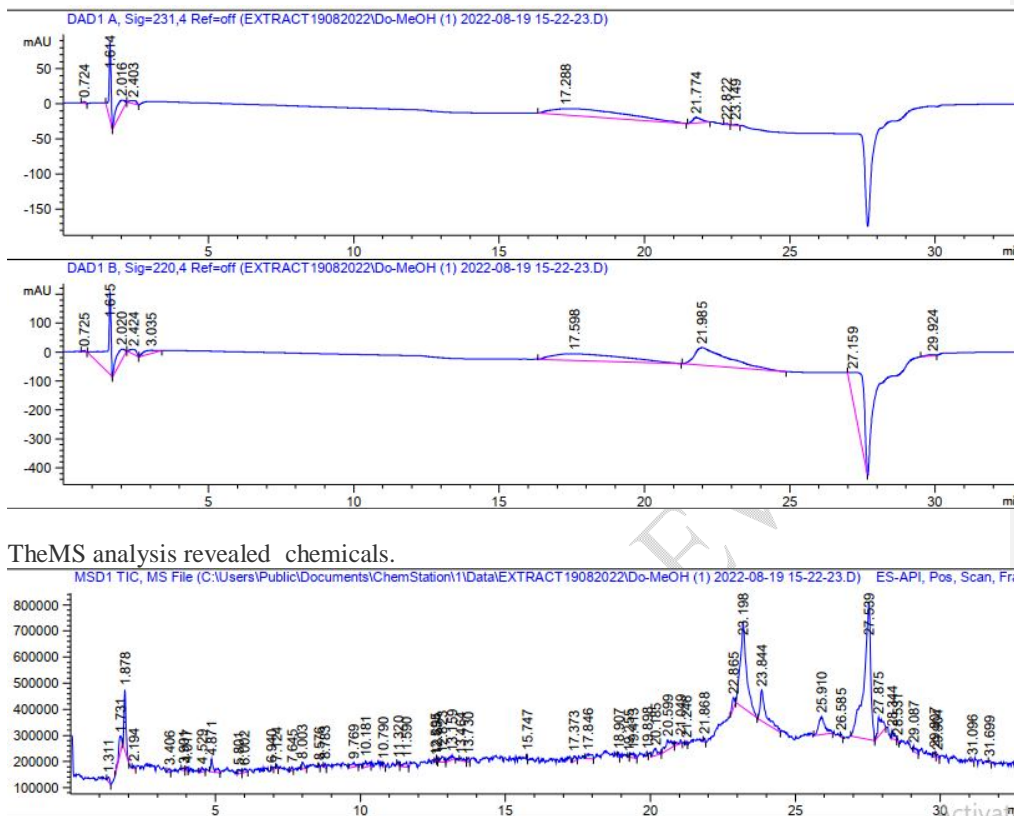
Parameter	Quantity (mg/100g)
Alkaloids	1000.179 \pm 0.61
Saponins	100.674 \pm 0.43
Tannins	1000.738 \pm 0.61
Flavonoids	300.923 \pm 0.15
Steroids	200.665 \pm 0.07
Cyanogenic glycosides	NIL
Phenols	13400.604 \pm 14.83
Terpenoids	2200.436 \pm 4.87

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

Table 7: HPLC-MSMS Area and Molecular ion mass

	Retention Time (Mins)	MS Area	Mol. IonWeight
1	1.311	86551	164.60
2	1.731	498026	164.70
3	1.878	1129825	207.80
4	2.194	40838	164.65

5	3.406	71248	164.70
6	3.901	40813	148.60
7	4.017	43136	148.65
8-	4.529	102369	148.65
9	4.871	359326	148.60
10	5.801	18425	148.65
11	6.002	97126	148.60
12	6.940	32544	148.65
13	7.124	39391	148.60
14	7.645	56779	148.60
15	8.003	146997	148.65
16	8.576	21873	148.70
17	8.783	44993	148.65
18	9.769	191973	148.70
19	10.181	233942	148.60
20	10.790	65188	148.60
21	11.320	53254	148.65
22	11.590	147038	148.65
23	12.595	17863	148.70
24	12.654	13476	148.65
25	12.595	17863	148.70
26	12.654	13476	148.65
27	12.823	187035	209.85
28	13.159	193081	148.65
29	13.464	84587	148.70
30	13.730	59494	148.60
31	15.747	26555	148.60
32	17.373	129673	148.65
33	17.846	223343	148.70
34	18.907	48233	186.70
35	19.255	14688	186.80
36	19.413	154260	186.75
37	19.898	18128	200.70
38	20.185	250245	206.70
39	20.599	532107	208.70
40	21.049	116592	208.70
41	21.246	28921	208.75
42	21.868	47793	208.70
43	22.865	228583	206.70
44	23.198	4906362	206.70
45	23.844	1575403	206.70
46	25.910	1025352	209.85
47	26.585	29067	208.80
48	27.539	8568353	148.65
49	27.875	770918	148.65
50	28.344	209444	148.65
51	28.531	13221	148.65
52	29.087	140218	148.65
53	29.807	71685	148.65
54	29.904	42564	148.65
55	31.096	26727	148.70
56	31.699	35378	148.65



TheMS analysis revealed chemicals.

Fig 3:HPLC Chromatogram

Discussion

According to the latest report, there were an estimated 241 million cases and 627 000 deaths globally in 2020 [2]. The ACTs are remarkable malaria treatments that have activity against both asexual blood-stage and sexual stages of parasite; however, the ACTs do not clear the latent liver stages of parasite. ACTs are required to demonstrate clinical efficacy of >95% polymerase chain reaction (PCR). Resistance to the artemisinins is characterized by a reduced rate of parasite clearance and create opportunity for development of resistance. There is concern that resistance is spreading. An increased prevalence, severity, or both of artemisinin resistance would be an enormous setback in the efforts to end malaria. With this in mind, novel approaches to accelerate the development efforts of new malaria therapeutics are urgently needed [30]. Results of the two endpoints in antimalarial assay % parasitemia suppression (>90%), and mean survival time (excess 5), are impressive and encouraging, indicating a great potential of the plant as a source of novel antimalarial agents are impressive.

Malaria patients should be checked for the presence of hematological abnormalities such as anemia and have to be managed for those abnormalities. Prediction of the hematological changes in malaria enables the clinician to establish an effective and early therapeutic intervention in order to prevent the occurrence of major complications. Hematology parameters can help to provide a presumptive treatment, especially when the results of the

Comment [a5]: In this discussion it is not explained why *Plasmodium berghei* is used in this test even though *Plasmodium berghei* does not infect humans (but *Plasmodium falciparum*, *ovale*, *vivax*, *malariae*, *knowlesi*). It is also not explained the possible mechanism of action of the plant/test material in suppressing the growth of plasmodium. The use of drugs in group 3 was not explained which in fact was given to *Plasmodium alciiparum*, *ovale*, *vivax*, *malariae*, *knowlesi* infections. It is also not explained the relationship between the content of the metabolites produced by this mechanism.

parasitological examination are not immediately available or are uncertain to decide treatment for malaria [31] and help to intensively care for the patient and prevent a death that may result from such complications[34].

In this research hematological parameters showed significant difference between the extract treated groups and the untreated group. The mean values of %PCV, RBC, HB, lymphocyte level, and WBC, were significantly lower in the untreated group compare to the standard drug and extract treated groups while thrombocyte and neutrophil count were elevated. This is in agreement with the work of Waitumbi et al., 2010 [35].

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 by [17] on the chemical composition of the oleoresin from *Daniellia oliveri* (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.

Efforts towards prevention of malaria are increasing with positive and promising results. The malaria vaccine RTS,S/AS01 was launched in 2021. Two vaccine candidates are approaching late-stage clinical evaluation: the R21/MatrixM vaccine targeting PfCSP protein and the attenuated whole sporozoite vaccine PfSPZ. Additional candidates targeting other malaria life-cycle stages include the Rh5 blood-stage vaccine candidate [36] and Pfs25 and Pfs230 vaccine candidates targeting sexual-stage antigens to prevent human-to-mosquito transmission [37]. New technologies, such as DNA- and mRNA-based vaccines [38,39], the ongoing development of adjuvants and delivery platforms such as virus-like particles (VLPs) [32] and vesicle-based technologies, are being explored for use in malaria vaccines [40].

Conclusion

Daniella oliveri bark methanol extract contains many secondary metabolites and has “High” anti-plasmodial activity, 81.1% reduction in parasitemia at a dose 600mg/kg body weight. A dose dependent pattern was observed. Mean survival time was excess 5. The 600 mg/Kg B. wt had 96 % the activity of the standard. *Daniella oliveri* is a “good” human antimalarial medicinal plant.

Comment [a6]: Keep in mind that *Plasmodium berghei* does not infect humans, so this statement is not correct

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