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Original Research Article

Antiplasmodial Potential of *Thaumatococcus danielli* Extracts (Benth.) against *Plasmodium berghei* Infected Mice

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ABSTRACT

Elimination of malaria infection with available antimalarial drugs is threatened by resistance developed by *Plasmodium* species and requires more efficacious and potent drugs. Some rural communities in Nigeria, use *Thaumatococcus danielli* leaves to cure malaria. However, a scientific investigation has not supported this assertion. In this investigation, swiss albino mice were used to test *T. danielli* extracts' antiplasmodial efficacy. The plant was subjected to qualitative phytochemical analysis, sohxlet extraction using n-hexane, ethyl acetate, ethanol, and aqueous solvents, including the extracts' acute oral toxicity study. Suppressive test of each extract against *Plasmodium berghei* (NK-65 strain) was tested at 200, 400 and 800mg/kg. Parasitaemia levels, temperature, weight, packed cell volume, and mean survival time of the mice were monitored during the study. In Windows Statistical Package for Social Sciences (SPSS) 26.0., one-way analysis of variance and Tukey's post hoc test were used to analyze the data. The acute oral toxicity study disclosed that LD_{50} values of *T. danielli* extracts were above 2,000mg/kg in mice. The maximum suppressive effect of 67.3% was achieved in a dose-dependent pattern ($p < 0.001$) by an oral administration of ethyl acetate extract at 800 mg/kg body weight. The extract was an ideal antiplasmodial extract since it minimized the fall in red blood cells, body temperature, and caused increased weight ($p > 0.05$). The plant has considerable antimalarial efficacy caused by individual or synergistic action of its phytochemicals. Therefore, bioassay of isolated and characterized active compound(s) is necessary for development of new antimalarial(s) from *T. danielli* leaves.

Keywords: *In vivo*, Malaria, *Plasmodium berghei*, *Thaumatococcus danielli*, Suppressive test

Introduction

Malaria is still a global health issue causing high morbidity and mortality across tropical and subtropical countries particularly sub-Saharan Africa (SSA).[1] The disease is caused by the transmission of the protozoan parasite, *Plasmodium* species by the female Anopheles mosquito to suitable hosts such as humans.[2] Nigeria accounts for the highest cases and death with *P. falciparum*, the most virulent species accounting for 95% of the country's infections. Infection is also holoendemic in the most vulnerable groups, children aged 0-5 years and pregnant women.[2]

The global decline in malaria cases has been attributed to the efficacy of control strategies, including the use of Artemisinin Combination Therapies (ACTs) as treatment.[3] Unfortunately, ACT, which is the foundation for malaria elimination, is becoming obsolete due to the development of resistance in *Plasmodium* species, particularly *Plasmodium falciparum*, to existing antimalarials.[3] Despite global evidence of reduced efficacy,[4,5] ACTs remain the priority treatment for uncomplicated malaria in Nigeria.[6]

Medicinal plants are a common alternative treatment for malaria in Nigeria used by traditional healers and the population.[7]

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This underscores the continuous screening of antimalarial plants for novel compound(s). Moreover, *Cinchona* species and *Artemisia annua* are medicinal plants that antimalarial compounds, artemisinin, and quinine were isolated from.[7] *Thaumatococcus danielli* Benn. (Benth) is a rhizomatous, perennial, and monocotyledonous plant that belongs to the family Marantaceae. In southwestern Nigeria, it is called Aba, Afremo, ewe moimoi, ewe eran. Alternative names are sweetener plant, sweet prayers plant, and katempe depending on its location.[8,9,10] It has long, slender stalks, two to three meters high, with a single hard, ovoid-shaped leaf of varying sizes, native to West Africa, especially Nigeria, Ghana, and Cote d'Ivoire.[8,10] The global distinction of the plant is consequent to the discovery of "thaumatin", a non-caloric sweetener derived from its arils and reportedly 1600 times sweeter than sucrose. The plant also has an economic contribution, especially in South Western Nigeria where it is mostly used in food wrapping.[9,10] Parts of plants are used to make mats, baskets, thatching roofs, wrapping food, sweetening food and drinks[8] as well as folkloric malaria treatments.[11,12] *T. danielli* extracts are also recognized for antioxidant activities,[8,10,13] protective effect in streptozotocin-induced diabetic rats,[12] protective effects on rats induced with potassium bromate testicular toxicity,[14] insecticidal activities of their essential oil,[13] antimicrobial and antibacterial activities.[15,16] This study investigated the acute toxicity, phytochemicals, and *in vivo* antiplasmodial activity of *Thaumatococcus daniellii*.

Materials and Methods

Test plant and phytochemical analysis

The green leaves of *Thaumatococcus danielli* were collected on 2nd June 2021 from Ishagira Island, Ojo local government area (N6º 26´ 29, E3º 07´ 40) of Lagos State and cleaned with tap water.[17] The plant was

authenticated with voucher number LSH001033 by Dr. O.K. Oluwa, a taxonomist in the Department of Botany, Lagos State University, Nigeria. Dried leaves of *T. danielli* were tested for phytochemicals according to the previously described standard procedures.[18]

Extraction of Thaumatococcus danielli leaves
The soxhlet extraction method was used to produce four distinct extracts using distilled water as the aqueous solvent as well as pure and analytically graded solvents of n-hexane, ethyl acetate, and ethanol. Thirty grammes (30 g) of dried and ground sample was used per extraction cycle. It was poured into the thimble of the soxhlet apparatus and extraction temperature was 55°C for all solvents except aqueous solvent that had its temperature at 70°C. The bioactive components of the leaves were extracted until the solvent in the siphon tube was clear. Solvents were evaporated from n-hexane, ethyl acetate, ethanol extracts in an air-dry oven at 37ºC and at 40°C for aqueous extract. All extracts were stored in glass bottles at -20ºC until needed.

Ethical approval for animal experimentation
The use of animals for this study was approved by the Institutional Review Board- Nigerian Institute of Medical Research (IRB-NIMR), Lagos, Nigeria with reference number IRB/17/028. The aforementioned institute's Animal House provided female Swiss albino mice. All mice were housed at room temperature with food and water *ad libithum* and acclimatized for a week before the study commenced. The use of animals in experiments was governed by international standards and laws.[19]

Acute oral toxicity test
Extracts of the test plant were assessed for their toxicity in agile female mice aged six to eight weeks; 19.4-21.8 grams (mean weight ± 2.0g/ group). Three female mice were assigned to each extract and control group. All animals fasted for three hours followed by oral administration of each extract at 2000 mg/kg body weight to determine the lethal dose (LD_{50}). Mice were monitored for observable changes and mortality within four hours of administration, followed by 24 hours till the 14th day.[20]

Malaria parasite and inoculation of test mice
Plasmodium berghei (NK-65 strain) sensitive to chloroquine phosphate was obtained from the Department of Biochemistry and Nutrition, NIMR, Lagos, Nigeria. Donor mice (24-27%) were passaged once with thawed parasites in the early hours of the day and bled after 72 hours for parasitized blood by puncture of the retroorbital sinus with a hematocrit tube into a heparinized tube.[21] To inoculate each mouse with $1x10^7$ *P. berghei*-infected red blood cells, one-tenth milliliter was aliquot from a mixture of three milliliters of physiological buffer saline (PBS) and one-tenth millimeter of parasitized blood.

Design for suppressive test
Seventy (70) female animals aged 6 to 8 weeks; 18.41-24.40 grams (mean weight ± 2.0g/ group) were randomly grouped into fourteen with 5 mice per group. Three groups of animals were allotted to each extract to test their efficacy against *P. berghei* at 200, 400, and 800 mg/kg per body weight of extract in mice. The positive control group was administered with 0.1 milliliter of 50 mg/kg analytical grade of chloroquine phosphate powder dissolved in 10 milliliters of distilled water[22] and the negative control group was left untreated. All animals were administered orally with the designated dose of extract/ drug after two hours of parasite inoculation on the first day (day 0). Treatment was consecutive for three days and on the last day (day 4), the tail was snipped to make thin blood smears on glass slides. Dried blood smears were fixed with methanol, stained with three percent Giemsa stain for 45 minutes and viewed a with x100 objective lens of the microscope to derive the parasitemia count in animals.[23] Ten random fields were examined to determine average parasitaemia levels in treated and untreated mice.

Percentage parasitemia and Percentage suppression were estimated as:[24]

$$\underset{Parasitaemia}{Percentage} = \frac{Number\ of\ infected\ red\ blood\ cells}{\underset{(infected\ and\ non-infected)}{Number\ of\ red\ blood\ cells}} \times 100$$

$$\underset{Suppression}{Percentage} = \frac{\left(\underset{negative\ control\ group}{Mean\ parasitemia\ of}\right) - \left(\underset{of\ extract\ treated\ group}{Mean\ parasitemia}\right)}{\underset{negative\ control\ group}{Mean\ parasitemia\ of}} \times 100$$

Change in body weight, temperature, and packed cell volume of mice
Weight, temperature, and packed cell volume were measured on day 0 (before parasite inoculation) and day 4 (after complete treatment). The mouse was weighed (grams) on a digital weighing balance (Mettler Toledo) and grouped within a mean ± 2.0g. The temperature (degree Celsius) was determined with the sensitive end of the digital thermometer in each mouse's rectum. The end of each procedure was indicated by a beep sound. The packed cell volume (percentage) of blood filled into three-quarters of hematocrit tubes was determined by spinning the tubes at 12,000 revolutions per minute (rpm) (Hawksley-Haematospin 1400) for 5 minutes and reading on a hematocrit reader (Tomy Seiko). Blood was collected by retroorbital sinus puncture.[21]

Evaluation of mean survival time (MST)
The animals in each group were monitored from the first day of the suppressive test until mortality occurred within 30 days.[25]

$$MST = \frac{Sum\ of\ days\ of\ survival\ of\ animals\ per\ group}{Total\ animals\ in\ the\ group}$$

Statistical analysis
Results of parasitemia, changes in body weight, temperature and packed cell volume also mean survival time were analysed utilizing SPSS software version 26.0 and detailed as mean ± standard error (mean ± SEM) for each treatment group. To analyze differences between groups and subgroups/within groups, respectively, one-way analysis of variance (ANOVA) and Tukey post hoc tests were used. When p is less than 0.05, differences were regarded as statistically significant.

Results and Discussion

Qualitative analysis of phytochemicals of *T. danielli* leaves detected alkaloids, anthraquinones, coumarins, flavonoids, saponins, sterols, and steroids as its secondary metabolites. These phytochemicals except coumarins and sterols were confirmed in another report.[9] Major classes of phytochemicals known to have antimalarial activities are phenolics, terpenoids, and alkaloids.[24,28] Tannins, flavonoids, coumarins, and phlobatannins[26] as well as anthraquinones[27] are derivatives of phenolics that were tested for and present in leaf of *T. danielli*.
The acute oral toxicity test of *T. danielli* extracts conducted at 2,000 mg/kg body weight did not cause physiological changes such as shivering, weak mobility, abnormal fecal secretion, hair erection or mortality within 24 hours till the 14th day after administration of extracts. This suggests that oral LD_{50} of the extract is beyond 2,000 mg/kg and safe for use in animals.[20]
Consequent to the account of an *in vivo* study in potential role of the immune system in infection eradication, it was used in this work to screen the plant extracts for their anti-plasmodial activity.[20] This method has shown that the immune system of the mice was unable to clear the parasite before administration of extract thereby causing malaria in infected animals and allowing for true account of effect of drug/extract on parasites.
Ethyl acetate administered groups had dose dependent pattern in chemo suppression of parasite. Mice treated with 200, 400 and 800 mg/kg body weight have shown parasitaemia suppression of 9.05%, 16.54% and 67.37% respectively. The effect of the highest dose was significant ($p < 0.001$) compared to the negative control (Table 1). Aqueous and ethanol extracts were not dose dependent in their actions, but at 800 mg/kg body weight, both extracts caused statistically significant suppression of parasites at 62.01% and 66.59%, respectively. Doses of n-hexane extract administered to the mice had an inverse proportional effect with the highest suppression of 54.30% at 200 mg/kg body weight which is significant ($p < 0.01$) compared to the negative control. The baseline drug, chloroquine phosphate, caused 100% clearance of parasites in mice.

The mean survival time of mice treated with 800 mg/kg of ethyl acetate and ethanol extracts also 200 mg/kg of n-hexane extract were longer than the negative control ($p > 0.05$). The survival period of mice in treated groups were much lesser compared to that of baseline drug (chloroquine 50 mg/kg) group that was tremendously ($p < 0.001$) prolonged compared to the negative group (Table 1). Parasite suppression and extension of survival time of mice compared to the negative group are reliable parameters in *in vivo* studies. Extracts are thus considered active when reduction in parasitemia is ≥30%.[28] All doses of aqueous and ethanol extracts, n-hexane extract at 200mg/kg and ethyl acetate extract at 800mg/kg performed above the stated benchmarks. Antiplasmodial activities could result from individual or combined action of phytochemicals present in the *T. danielli* extracts.[24] These results affirm indigenous use of *T. danielli* for malaria treatment by traditional healers and people of Ishagira Island, Lagos State. An ethnobotanical survey for antimalarial plants in Badagry local government area, Lagos State which is in proximity to Ishagira Island also documented use of *T. danielli* for malaria treatment.[11]

Table 1: Suppressive effect and mean survival time of Aqueous, Ethanol, Ethyl acetate, and n-Hexane extracts of *T. danielli* leaves

Solvent	Dosage (mg/kg)	Percentage parasitemia	% Suppression	Mean survival time (Days)
	200	50.9 ± 0.69	43.13^{a3b3}	9.8 ± 0.86^{b3}
Aqueous	400	57.8 ± 0.91	35.42^{a1b3}	7.6 ± 0.68^{b3}
	800	34.0 ± 0.53	62.01^{a3b2}	10.2 ± 0.86^{b3}
	200	43.2 ± 0.39	51.73^{a3b3}	9.6 ± 1.50^{b3}
Ethanol	400	54.9 ± 0.83	38.66^{a3b3e1}	10 ± 1.79^{b3}
	800	29.9 ± 0.58	66.59^{a3b2d1}	11.2 ± 2.2^{b3}
	200	40.9 ± 0.64	54.30^{a2b1}	10.8 ± 1.16^{b3}
n-Hexane	400	75.4 ± 1.66	29.16^{b3}	10 ± 1.58^{b3}
	800	63.4 ± 0.2	15.75^{b3}	9.6 ± 1.21^{b3}
	200	81.4 ± 1.1	9.05^{b3e3}	9.4 ± 1.33^{b3}
Ethyl acetate	400	74.7 ± 0.75	16.54^{b3e2}	10.6 ± 1.75^{b3}
	800	29.2 ± 1.17	67.37^{a3c3d2}	11.8 ± 1.39^{b3}
Chloroquine	50	0	100^{a3}	26.6 ± 1.72^{a3}
Negative control	0	89.5 ± 0.68	0.00	10.6 ± 2.77

Values presented as mean ± SEM; n = 5; a, comparison with negative control; b, chloroquine 50mg/kg; c, 200 mg/kg; d, 400 mg/kg; e, 800 mg/kg; [1] =$p<0.05$; [2]=$p<0.01$;[3]=$p<0.001$

Tested extracts at varying doses induced insignificant weight gain ($p > 0.05$) in treated mice (Tables 2-5), except 200 and 400 mg/kg ethyl acetate extract (Table 5), which could not prevent weight loss. The standard drug (chloroquine 50 mg/kg) caused tremendous weight gain ($p < 0.001$) in comparison to the negative control and extract treated groups (Tables 2-5). The standard drug also attenuated fall in temperature ($p < 0.001$) and PCV ($p < 0.001$) in its treated mice compared to the negative control and extract treated groups (Tables 2-5). However, the *T. danielli* extracts were unable to attenuate the decrease in temperature (Tables 2-5), except for the ethanol extract at 800 mg/kg ($p > 0.05$) compared to the negative control (Table 3). Likewise, the extracts did not prevent a reduction in PCV compared to the negative control (Tables 2-5) except at 800 mg/kg body weight of ethyl acetate extract ($p > 0.05$) (Table 5). Red blood cell reduction, body weight, and temperature are relative symptoms of malaria in infected mice.[29] An ideal anti-malarial compound is expected to prevent fall in body weight, temperature, and PCV in mice.[28,29] In this study, the ethyl acetate extract at 800mg/kg body weight appears to be the most ideal extract. In addition to having the highest suppression and survival time, the extract was able to minimize reduction in PCV, body temperature, and cause an appreciable increase in weight amongst other treated groups (Table 5).

Conclusion

Findings of this study revealed antiplasmodial activities of crude extracts of *T. danielli* with ethyl acetate extract being the most active and ideal of them. The anti-malarial activities of the crude extract could be attributed to the single or combined effect of phytochemicals with established antiplasmodial potentials. This study therefore is a proof of the indigenous claims of antiplasmodial activities of *T. danielli* by traditional healers and the Nigerian population. Furthermore, studies towards isolation and characterization of active compound(s) of *T. danielli* are needed for drug development.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

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Table 2: Percentage changes in weight, temperature and packed cell volume of *P. berghei* infected mice treated with aqueous extract of *T. danielli* leaf

Solvent	Dosage (mg/kg)	Weight (g)		% Change	Temperature (ºC)		% Change	Packed cell volume (%)		% Change
		Day 0	Day4		Day 0	Day4		Day 0	Day4	
Aqueous	200	24.4 ± 0.09	25.68 ± 0.13	5.22 ± 0.27	36.68 ± 0.29	36.16 ± 0.07	-1.39 ± 0.75	48.2 ± 0.86	39.3 ± 0.54	-18.44 ± 0.57[b3]
	400	19.45 ± 0.25	21.21 ± 0.26	9.05 ± 0.99	36.48 ± 0.24	36.24 ± 0.06	-0.64 ± 0.77	49.4 ± 0.68	40 ± 1.14	-19.05 ± 1.79[b3]
	800	19.2 ± 0.31	20.17 ± 0.26	5.10 ± 0.93	36.64 ± 0.19	36.24 ± 0.26	-1.08 ± 0.88	47.3 ± 1.59	38.6 ± 1.21	-18.33 ± 1.21[b3]
Chloroquine	50	21.15 ± 0.24	23.7 ± 0.32	12.08 ± 1.81[a3]	36.68 ± 0.15	36.9 ± 0.12	0.6 ± 0.56[a3]	42.8 ± 0.97	41.6 ± 0.68	-2.58 ± 2.9[a3]
Negative Control	0	20.59 ± 0.42	20.23 ± 1.08	-1.52 ± 6.03	37.1 ± 0.13	36.44 ± 0.54	-1.78 ± 1.34	47 ± 0.89	39.4 ± 0.93	-16.19 ± 0.6

Data represented as mean ± SEM; n = 5; a, compared to negative control; b, chloroquine 50mg/kg; c, 200 mg/kg; d, 400 mg/kg; e, 800 mg/kg; [1]=p<0.05; [2]=p<0.01; [3]=p<0.001

Table 3: Percentage changes in weight, temperature and packed cell volume of *P. berghei* infected mice treated with ethanol extract of *T. danielli* leaf

Solvent	Dosage (mg/kg)	Weight (g)		% Change	Temperature (ºC)		% Change	Packed cell volume (%)		% Change
		Day 0	Day4		Day 0	Day4		Day 0	Day4	
Ethanol	200	20.34 ± 0.38	21.43 ± 0.48	5.32 ± 0.43	36.86 ± 0.27	36.2 ± 0.12	-1.78 ± 0.6	45.66 ± 0.98	35.9 ± 0.64	-21.33 ± 0.87[b3]
	400	22.64 ± 0.4	23.39 ± 0.36	3.33 ± 0.35	36.6 ± 0.2	36.26 ± 0.11	-0.92 ± 0.64	47.44 ± 1.01	38 ± 0.65	-19.86 ± 0.63[b3]
	800	20.45 ± 0.38	22.11 ± 0.47	8.08 ± 0.35	36.46 ± 0.05	36.48 ± 0.09	0.06 ± 0.22	47 ± 0.71	36.6 ± 0.75	-22.12 ± 1.22[b3]
Chloroquine	50	21.15 ± 0.24	23.7 ± 0.32	12.08 ± 1.81[a3]	36.68 ± 0.15	36.9 ± 0.12	0.6 ± 0.56[a3]	42.8 ± 0.97	41.6 ± 0.68	-2.58 ± 2.9[a3]
Negative Control	0	20.59 ± 0.42	20.23 ± 1.08	-1.52 ± 6.03	37.1 ± 0.13	36.44 ± 0.54	-1.78 ± 1.34	47 ± 0.89	39.4 ± 0.93	-16.19 ± 0.6

Data represented as mean ± SEM; n = 5; a, compared to negative control; b, chloroquine 50mg/kg; c, 200 mg/kg; d, 400 mg/kg; e, 800 mg/kg; [1]=p<0.05; [2]=p<0.01; [3]=p<0.001

Table 4: Percentage changes in weight, temperature and packed cell volume of *P. berghei* infected mice treated with n-hexane extract of *T. danielli* leaf

Solvent	Dosage (mg/kg)	Weight (g)		% Change	Temperature (ºC)		% Change	Packed cell volume (%)		% Change
		Day 0	Day4		Day 0	Day4		Day 0	Day4	
n-hexane	200	22.23 ± 0.18	23.56 ± 0.31	5.97 ± 0.81	36.64 ± 0.27	36.18 ± 0.09	-1.23 ± 0.86	40.8 ± 1.24	33.94 ± 1.43	-16.91 ± 1.2[b3e1]
	400	24.34 ± 0.39	25.83 ± 0.39	6.15 ± 0.45	36.72 ± 0.33	36.26 ± 0.05	-1.22 ± 0.92	46.8 ± 1.07	36.3 ± 0.49	-22.36 ± 0.97[b3]
	800	22.67 ± 0.33	22.93 ± 0.28	1.17 ± 0.6	36.54 ± 0.27	36.14 ± 0.14	-1.08 ± 0.48	47.7 ± 0.58	36 ± 0.35	-24.51 ± 0.75[a2b3]
Chloroquine	50	21.15 ± 0.24	23.7 ± 0.32	12.08 ± 1.81[a3]	36.68 ± 0.15	36.9 ± 0.12	0.6 ± 0.56[a3]	42.8 ± 0.97	41.6 ± 0.68	-2.58 ± 2.9[a3]
Negative Control	0	20.59 ± 0.42	20.23 ± 1.08	-1.52 ± 6.03	37.1 ± 0.13	36.44 ± 0.54	-1.78 ± 1.34	47 ± 0.89	39.4 ± 0.93	-16.19 ± 0.6

Data represented as mean ± SEM; n = 5; a, compared to negative control; b, chloroquine 50mg/kg; c, 200 mg/kg; d, 400 mg/kg; e, 800 mg/kg; [1]=p<0.05; [2]=p<0.01; [3]=p<0.001

Table 5: Percentage changes in weight, temperature and packed cell volume of *P. berghei* infected mice treated with ethyl acetate extract of *T. danielli*

Solvent	Dosage (mg/kg)	Weight (g)		% Change	Temperature (ºC)		% Change	Packed cell volume (%)		% Change
		Day 0	Day4		Day 0	Day4		Day 0	Day4	
Ethyl acetate	200	18.41 ± 0.21	16.16 ± 0.31	-12.17 ± 2.07^{b3e3}	37.04 ± 0.33	35.86 ± 0.27	-3.18 ± 0.43	48.3 ± 0.88	39.4 ± 0.93	-18.37 ± 1.98^{b3}
	400	19.93 ± 0.37	19.43 ± 0.84	-2.30 ± 4.99 ^{b1}	36.76 ± 0.28	36.18 ± 0.38	-1.56 ± 1.24	44.8 ± 1.66	37.5 ± 1.22	-16.21 ± 1.21^{b3}
	800	20.28 ± 0.35	22.03 ± 1.11	8.73 ± 5.64^{c3}	36.7 ± 0.31	36.66 ± 0.46	-0.07 ± 1.62	47.2 ± 1.07	40.5 ± 1.07	-14.16 ± 1.72^{b3}
Chloroquine	50	21.15 ± 0.24	23.7 ± 0.32	12.08 ± 1.81^{a3}	36.68 ± 0.15	36.9 ± 0.12	0.6 ± 0.56^{a3}	42.8 ± 0.97	41.6 ± 0.68	-2.58 ± 2.9^{a3}
Negative Control	0	20.59 ± 0.42	20.23 ± 1.08	-1.52 ± 6.03	37.1 ± 0.13	36.44 ± 0.54	-1.78 ± 1.34	47 ± 0.89	39.4 ± 0.93	-16.19 ± 0.6

Data represented as mean ± SEM; n = 5; a, compared to negative control; b, chloroquine 50mg/kg; c, 200 mg/kg; d, 400 mg/kg; e, 800 mg/kg; ^{1}=p<0.05; ^{2}=p<0.01; ^{3}=p<0.001

References

1. Cowman AF, Healer J, Marapana D, Marsh K. Malaria: Biology and Disease. Cell. 2016; 167(3):610-624.
2. Okwa OO. Nipping the Malaria Vectors in the Bud: Focus on Nigeria. Intech Open Access Publisher Fyson Kasenya (Ed). 2019; Chapter 5:91-102.
3. Dhiman S. Are malaria elimination efforts on right track? An analysis of gains achieved and challenges ahead. Infect Dis Poverty. 2019; 8(14):1-19.
4. Ajayi NA, Ukwaja KN. Possible artemisinin-based combination therapy-resistant malaria in Nigeria: a report of three cases. Rev Soc Bras Med Trop. 2013; 46(4):525-527.
5. Takala-Harrison S, Jacob CG, Arze C, Cummings MP, Silva JC, Dondorp AM, *et al*. Independent emergence of artemisinin resistance mutations among *Plasmodium falciparum* in Southeast Asia. J Infect Dis. 2015; 211(5):670-679.
6. Welle SC, Ajumobi O, Dairo M, Balogun M, Adewuyi P, Adedokun B, Nguku P, Gidado S, Ajayi I. Preference for Artemisinin-based combination therapy among healthcare providers, Lokoja, North-Central Nigeria Glob Health Res Policy. 2019; 4(1):1-8.
7. Adebayo JO, Krettli AU. Potential antimalarials from Nigerian plants: A review. J Ethnopharmacol. 2011; 133(2):289-302.
8. Adu OB, Adeyemo, GA, Falua, OB, Fajana, OO, Ogunrinola, OO, Saibu, GM, Elemo, BO. The effect of *Thaumatococcus danielli* leaf extracts on immunological and oxidative stress markers in rat. AJBGMB. 2021; 7(4):6-14.
9. Ojekale AB, Makinde, SCO, Osileye, O. Phytochemistry and anti-microbial evaluation of *Thaumatococcus danielli*, Benn. (Benth.) leaves. NIFOJ. 2007; 25:176-183.
10. Ayodeji OI, Adeleye O, Dada O, Adeyemi O, Anyasor GN. Phytochemical constituent and antioxidant activity of *Thaumatococcus daniellii* Benn (Benth.) leaves (Food Wrapper). IJPPE. 2016; 2:55-61.
11. Adedeji DE, Kayode J, Ayeni MJ. An ethnobotanical study of plant species used for medicine by the Eegun indigenous tribal group of Lagos State, Nigeria. Not Sci Biol. 2018; 10(3):318-327.
12. Olorunnisola OS, Adetutu A, Popoola RB, Owoade AO, Adegbola P, Adesina BT. Nephroprotective effect of ethanolic leaf extract of *Thaumatococcus danielli* (benth.) in streptozotocin induced diabetic Rats. FFHD. 2017; 7(12):923-935.
13. Ojekale AB, Lawal OA, Segun AA, Samuel FO, Ismaila AI, Opoku AR. Volatile constituents, antioxidant and insecticidal activities of essential oil from the leaves of *Thaumatococcus danielli* (Benn.) Benth. from Nigeria. IOSR J Pharm. 2013; 3(3):1-5.
14. Nwonuma CO, Irokanulo EO, Iji CE, Alejolowo OO, Adetunji CO. Effect of *Thaumatococccus daniellii* leaf rat-feed on Potassium bromate induced testicular toxicity. Asian Pacific J Rep, 2016; 5(6):500-505.
15. Hamid AA, Aliyu MA, Abubakar LZ, Mukadam AA, Shehu A, Egharevba G, Adisa MJ, Ajibade SO, Zubair AO, Fagbohun EO. *Thaumatococcus daniellii* leaves: Its chemical compositions, antioxidant and antimicrobial activities. IJS, 2017; 19(2):409-416.
16. Ukwubile CA, Oise IE, Nyiayem JT. Preliminary phytochemical screening and antibacterial activity of *Thaumatococcus daniellii* (Benn.) Benth.(Marantaceae) leaf extract. J Bacteriol Mycol, 2017; 4(2):53-57.
17. Arshad M, Ullah MI, Afzal M, Khalid S, Raza AM, Iftikhar Y. Evaluation of plant extracts for the management of citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Kuwait J. Sci. 2019; 46(1):58-67
18. Khan AM, Qureshi RA, Ullah F, Gilani SA, Nosheen A, Sahreen S, Leghari MK, Laghari MY, Rehman S, Hussain I, Murad W. Phytochemical analysis of selected medicinal plants of Margalla Hills and surroundings. J Med Plant Res 2011; 5:6017-6023.
19. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG. Animal research: Reporting *in vivo* experiments: The ARRIVE guidelines Br J Pharmacol. 2010; 160(7):1577-1579.
20. Zeleke G, Kebebe D, Mulisa E, Gashe F. *In vivo* Antimalarial Activity of the Solvent Fractions of Fruit Rind and Root of *Carica papaya* Linn (Caricaceae) against *Plasmodium berghei* in Mice. J Parasitol Res. 2017; 3121050:1-10.
21. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother. 2010; 1(2):87-93.
22. Iribhogbe OI, Agbaje EO, Oreagba IA, Aina OO, Ota AD. Therapeutic potential of selected micronutrients in malaria: an *in vivo* study in *Plasmodium berghei* infected mice Biol Med. 2012; 4:193-201.
23. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery: Efficacy models for compound screening. Nat Rev Drug Discov. 2004; 3:509-520.
24. Ali BH, Mohammed I, Muhammed MA, Mohammed SY, Garba D, Busola OA, Khadijah II, Manager MM. Evaluation of *in vivo* antiplasmodial activity of the methanol

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root bark extract and fractions of *Bombax costatum* (Bombacacea) in *Plasmodium berghei*-infected mice. Trop J Nat Prod Res. 2022; 6(6):926-930.
25. Gebrehiwot S, Shumbahri M, Eyado A, Yohannes T. Phytochemical Screening and *In vivo* Antimalarial Activity of Two Traditionally Used Medicinal Plants of Afar Region, Ethiopia, against *Plasmodium berghei* in Swiss Albino Mice. J Parasitol Res. 2019; 4519298:1-10.
26. Ferreira JF, Luthria DL, Sasaki T, Heyerick A. Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. Molecules. 2010; 15(5):3135-3170.
27. Diaz-Munoz G, Miranda IL, Sartori SK, de Rezende DC, Diaz MAN. Anthraquinones: An Overview. In: Studies in Natural Products Chemistry. 2018; 58:313-338.
28. Misganaw D, Engidawork E, Nedi T. Evaluation of the anti-malarial activity of crude extract and solvent fractions of the leaves of *Olea europaea* (Oleaceae in mice. BMC Complement Altern Med. 2019; 19(171):1-12 .
29. Bantie L, Assefa S, Teklehaimanot T, Engidawork E. *In vivo* antimalarial activity of the rude leaf extract and solvent fractions of *Croton macrostachyus* Hocsht. (Euphorbiaceae) against *Plasmodium berghei* in mice. BMC Complement Altern Med. 2014; 14(79):1-10.