ORIGINAL RESEARCH ARTICLE

Antiplasmodial effects of aqueous leaf extracts of *Senna alata* and *Dennettia tripetalla* in chloroquine-sensitive *Plasmodium berghei berghei* (NK65) infected mice

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Abstract
The antiplasmodial effects of *Senna alata* and *Dennettia tripetalla* on chloroquine-sensitive *Plasmodium berghei berghei* (NK65) were analysed. *P. berghei* was obtained from National Institute for Medical Research, Lagos, Nigeria, and maintained in the laboratory by serial passage in mice. Chemosuppressive, prophylactic, and curative procedures were used to evaluate the antiplasmodial potentials of the extracts against established infection. A total of 320 albino mice were used for this study. Twenty-five albino mice were divided into 5 groups and used for *D. tripetalla* acute toxicity study. Eighty-five albino mice were divided into 15 groups of five albino mice each and used for chemosuppressive tests while two hundred and ten mice were used for prophylactic and curative tests. The infected mice were orally treated daily with 5mg/kg chloroquine 500 mg/kg, 1000 mg/kg, 1500 mg/kg, and 2000 mg/kg body weight of *S. alata, D. tripetalla,* and *S. alata + D. tripetalla respectively*. The *in vivo* antiplasmodial effect of the extracts against *P. berghei* infection showed a significant ($p<0.05$) dose-dependent schizontocidal activity for the chemosuppressive, and prophylactic test. Results of the curative study showed that the combination of both *S. alata* and *D. tripetalla* exhibited a strong curative effect on malaria parasitaemia. There was a significant clearance ($p<0.05$) in parasiteamia level from day 1 to day 5 of treatment in a dose-dependent manner with a corresponding significant ($p<0.05$) percentage suppression. It was therefore concluded that the leaf extracts of *S. alata* and *D. tripetalla* possess antimalarial potencies which could be exploited for antimalarial therapy.

Keywords: *Chemosuppressive, Prophylactic, Curative, Parasitaemia, Senna alata, Dennettia tripetalla, Plasmodium berghei*

INTRODUCTION

Malaria is an infectious disease caused by plasmodium parasites that invade the red blood cells (Adumanya *et al.,* 2014). It is characterized by fever, headaches, lethargy, tiredness, nausea, and anaemia which may appear on alternate days following malaria infection. Organ failure can also occur in severe cases of the illness (Mishra *et al.,* 2002). The plasmodium parasites which are transmitted to humans from mosquito bites continue to pose a global public health challenge (Daniels *et al.,* 2017). According to the World Health Organization, there were an estimated 241 million cases of malaria in 2020 worldwide. The estimated number of malaria deaths stood at 627 000 in 2020 – an increase of

69 000 deaths over the previous year. (WHO, 2021). In 2020 the African Region was home to 95% of all malaria cases and 96% of deaths. Children under 5 years of age accounted for about 80% of all malaria deaths in the Region, with Nigeria having the highest number of infections, accounting for nearly 31.9% of the entire global malaria burden. Incidentally, Children under the age of five, are the most vulnerable group to malaria. About 80% of all malaria-related deaths in 2020 were from this category (WHO, 2021). Thus, the occurrence of malaria in Nigeria is disproportionately high and calls for immediate action to prevent infant and maternal deaths.

Plasmodium falciparum is the most common etiological agent of malaria in sub-Saharan Africa. The malaria parasite has become more resistant to conventional antimalarial drugs including chloroquine, artemisinin, and sulfadoxine-pyrimethamine. Artemisinin Combination Treatments (ACTs) are also threatened with the emergence of resistant parasites though they are the preferred drug of choice against malaria infection. As a result, there are intense efforts globally to fight malaria infections. Two main approaches have been employed to do so: elimination of the plasmodium parasite and prevention of malaria disease. Although both strategies have been faced with challenges, significant efforts have been made to address malaria infections (Lover *et al.,* 2018). Some of the malaria prevention techniques include the use of insecticides and mosquito nets (Fairhurst *et al.,* 2012). However, the incidence of malaria globally is still high, especially in endemic areas due to challenges in following the complete malaria prevention techniques. Hence, no single preventive measure is 100% effective. Thus, a combination of various preventive strategies is now advised in the global fight to eliminate malaria (Wangdi *et al*., 2018).

Currently, artemisinin-based combination therapy (ACT) has been recommended by WHO for the treatment and prevention of malaria (Fairhurst *et al.,* 2012). According to some reports, due to socioeconomic challenges and the high cost of purchasing effective malaria drugs as a result of rising poverty rates, as well as the difficulty of obtaining ACT from Primary Health Care Centers, most people, particularly those in rural areas of Nigeria, have resorted to using herbs as an alternative remedy to combat malaria and other diseases (FMOH, 2005; Onwujekwe and Uzochukwu, 2005; FMOH, 2011). Additionally, about 80% of the population of developing countries is heavily reliant on herbal medicines and concoctions derived from plants for the treatment of diseases including malaria infections (Ekor, 2013; Oputah *et al.,* 2016). The easy accessibility of herbs and the minimal to no cost associated with getting these herbs are some of the key factors that contribute to making herbal medicines quite popular, especially in rural areas (Hussain *et al.,* 2018). Also, the belief in the deductive approach based on recommendation and the illogical belief that herbal medicines are more effective than orthodox pharmaceutical products have contributed to the increasing use of herbal drugs to tackle various illnesses (Ekor, 2013).

Although pharmaceutical drugs including Chloroquine Phosphate (Primaquine) are effective in the treatment of malaria, they also present some side effects which include nausea, blurred vision, pruritus, paraesthesia, and insomnia. Primaquine has haemolytic activities and results in discolouration of urine in people with glucose 6 phosphate dehydrogenase deficiency (Braga *et al.,* 2015). Side effects of Artemisinin-based Combination Therapy include mild hepatitis and haemolytic anaemia (Pousibet-Puerto *et al*., 2016). In many regions of the globe, medicinal plants have been the focus in the quest for novel drugs including antimalarial medicines (Schuster, 2001). Consequently, several herbal products are now used to tackle various ailments, and about 50,000 angiosperms are now used as medicines (Hussain *et al.,* 2018). More so, the use of herbs and plants is eco-friendly and in line with the guidelines for tackling climate change which is also a global challenge. Plants have continued to serve as an important source for the discovery of new drugs including drugs used for the

treatment of malaria. Artemisinin for example was obtained from *Artemisia annua* and quinine derived from *Cinchona offiinalis*. Therefore, it is now necessary to screen for novel plants with antiplasmodial potentials, and the subsequent identification and characterization of the active components in such plants would serve as useful pharmacological agents and guide towards the formulation of safer, cheaper, more affordable antiplasmodial drugs with reduced side effects and toxicity.

Additionally, the use of herbs for the treatment of diseases is an important contributory factor to primary health care, especially in the rural areas of developing countries (Hussain *et al.,* 2018) including Nigeria where access to affordable health care is quite difficult. Thus, herbal medicine is quite popular amongst rural dwellers and contributes significantly to the wellbeing of the rural population (Calixto, 2005; Hussain *et al.,* 2018).

Several plants used to treat malaria infection have been previously investigated. Some of them include *Salacia senegalensis* (Adumanya *et al.,* 2014), *Commiphora africana*, *Dichrostachys cinerea* (Kweyamba *et al.,* 2010), and *Strychnos mitis* (Fentahun *et al.,* 2017). Leaves of *S. alata* have been widely used to treat malaria among rural dwellers in Nigeria. *D. tripetalla* leaves (popularly called pepper fruit) have also been used to treat malaria, especially in southeast Nigeria. Although there are claims of how effective these leaves can be in the treatment of malaria infection, there are no scientific data to support these claims yet. Thus, this study has been designed to investigate the antiplasmodial potentials of aqueous leaf extracts of *S. alata* and *D. tripetalla* in treating malaria infections.

MATERIALS AND METHODS

Plant materials collection and authentication

Fresh leaves of *Senna alata* were obtained from UNIPORT botanical garden, Abuja campus, University of Port Harcourt, Rivers State, Nigeria. *Dennettia tripetalla* leaves were collected from farmland in Obohia-Ndoki in Ukwa-East LGA, Abia State. ‘The plant samples were authenticated and assigned voucher numbers by Dr Eke Chimezie of the Department of Plant Science and Biotechnology. The voucher specimen UPH/V/1225 for *S. alata* and UPH/V/1269 for *D. tripetalla* were deposited in the Plant Science and Biotechnology Herbarium of the University of Port Harcourt, River State.

Preparation of aqueous leaf extracts

The leaves of *S. alata* and *D. tripetalla* were cleaned, air-dried at room temperature (27°C) for 21 days, and pulverized with the aid of malex excellent grinder (Ugbogu *et al*., 2016). The aqueous extracts were prepared by soaking 1000g (Timothy *et al*., 2012) each of the dry powdered plant materials in 2500 litres of distilled water at room temperature for 72 hours (Ehiowemwenguan *et al*., 2014). The extracts were filtered after 72 hours using a ball of cotton wool and Whatman No. 42 filter paper (125 mm) and thereafter, concentrated into a powder form using a rotary evaporator with a water bath set at 40°C (Abdullahi *et al*., 2013). The concentrated extracts were collected in an airtight container and stored in a refrigerator at 4°C till use.

Animals

The experimental animals used for this study were healthy albino mice with weights between 22-26 grams. All experimental animals used for this study were obtained from the Animal House of the Department of Biochemistry, Faculty of Science, University of Port Harcourt. The animals were kept in cages in a well-ventilated room and provided with food and water *ad libitum*.

Acute oral toxicity test (LD_{50}) determination

Twenty-five (25) albino mice were divided into five groups of five mice each according to the method of Aliu and Nwude, (1982). Group 1 received 0.3 ml of water while Groups 2 - 5 received 500, 1000, 1500, and 2000 mg/kg bodyweight of *D. tripetalla* respectively. Administration of extract was done orally with a cannula attached to a graduated syringe. The mice were observed for 96 h for signs of toxicity.

Mice inoculation with *Plasmodium berghei* parasite

Chloroquine-sensitive *Plasmodium berghei berghei* (NK65)-infected mice were first obtained from National Institute for Medical Research, Lagos. Parasitaemia levels of donor mice were then determined by cutting the tail and taking thick blood films using a microscope slide, allowed to dry and stained with 10% Giemsa stain. Thereafter, immersion oil was added to the slide and viewed under the microscope using 40X magnification under the light microscope. A total of 500 Red Blood Cells (RBCs) were counted and the numbers of infected RBCs in the 500 RBCs were noted using a tally counter and percentage parasitaemia was calculated accordingly. The donor mice were then sacrificed and blood was collected by cardiac puncture in an EDTA bottle and 1ml of the donor blood was added to 5ml of physiological saline (0.9%). Then, 0.3ml of the diluted blood was administered intraperitoneally to each mouse.

Extract and drug administration

An orogastric tube was used to administer both the extracts and chloroquine phosphate which was obtained from Ikefrank Pharmacy, Woji, Port Harcourt, River State, Nigeria.

Experimental design and animal groupings

Chemosuppressive, prophylactic, and curative procedures were used to evaluate the antiplasmodial potentials of the aqueous extracts of *S. alata*, *D. tripetalla*, and a mixture of both *S. alata* and *D. tripetalla* leaf extracts in a ratio of 1:1. Chemosuppressive test measures the ability of the extract to suppress the infection rate of the parasite, while prophylactic and curative effects measure the activities of the extract against early residual and established infections respectively. The experimental design comprised of Group 1 - not infected mice and received only food and water also referred to as the normal control, Group 2 - infected mice and not treated, also referred to as the negative control, Group 3 - infected mice and treated with 5mg/kg chloroquine daily, and Groups 4 - 7 infected mice and treated with *S. alata*, Groups 8 – 11 infected mice and treated with *D. tripetalla*, Groups 12 - 15 infected mice and treated with a mixture of *S. alata* and *D. tripetalla* in a graded dose of 500 mg/kg, 1000 mg/kg, 1500 mg/kg, and 2000 mg/kg body weight respectively. Percentage parasitaemia levels were calculated using the formula described by Peters and Robinson (1992).

Curative test of *Plasmodium berghei berghei*

The mice were first inoculated using the blood of infected donor mice as described above. After 72 hours following inoculation, thick blood films were made by taking blood samples from the tails of the mice to measure the initial level of parasitaemia in the mice. Then, each of the extracts of *S. alata*, *D. tripetalla*, and a mixture of *S. alata* and *D. tripetalla* in a ratio of 1:1 according to the dosage group of 500 mg/kg, 1000 mg/kg, 1500 mg/kg, and 2000 mg/kg were administered for 5 consecutive days while taking thick films from the tails after every 24 hours following extract administration.

Prophylactic test of *Plasmodium berghei berghei*

The experimental mice were divided into 5 groups of 5 mice accordingly; 5mg/ kg of chloroquine phosphate was administered to one group while the remaining 4 groups received 500 mg/kg, 1000

mg/kg, 1500 mg/kg, and 2000 mg/kg respectively for four consecutive days. On the fifth day, the mice were inoculated as described above. Thick blood films were then obtained from the tails of the mice after 72 hours following inoculation and observed for the presence of the parasite.

Chemo-suppressive test of *Plasmodium berghei berghei*

The mice were first inoculated as described above. About one hour after inoculation, for each extract group, the mice were divided into 5 groups of 5 mice accordingly; 5mg/ kg of chloroquine phosphate was given to one group while the remaining 4 groups each received 500 mg/kg, 1000 mg/kg, 1500 mg/kg and 2000 mg/kg of the extracts for four consecutive days. Thin blood films were then made from the tails of mice to access the parasitaemia levels and chemo-suppressive potential of the extracts.

Microscopy techniques

The thick and thin blood films were made from the tails of the mice onto cleaned microscope slides which were then allowed to air dry for 45 minutes. For the thick film, the microscope slide was dipped into 10% Giemsa stain and subsequently washed by dipping in buffered distilled water for 5 minutes and allowed to dry. For the thin film, after the blood was taken from the tails of mice onto the microscope slides and allowed to air dry, the slides were then dipped three times into methanol and allowed to dry for 25 minutes. This was then flushed with tap water and allowed to dry. Immersion oil was then added to the slides and viewed under a light microscope. Using a hand tally, the parasitaemia levels were then determined by counting the number of parasitized RBCs from a total of 500 red blood cells (Ochei and Kolhatkar, 2000). Percentage parasitaemia was calculated using the formula;

$$\textbf{PP} = \frac{Total\ No.of\ PRBC}{No.\ of\ RBC} \ x \ \frac{100}{1}$$

Where, PP = Percentage parasitaemia, PRBC = Parasitized red blood cells, RBC=Red blood cells.

The percentage suppression of parasitaemia was then calculated with respect to the control group using the formula;

$$\textbf{A} = \frac{B-C}{B} \ x \ \frac{100}{1}$$

Where, A = average percentage suppression of parasitaemia, B = average percentage parasitaemia in the control group (distilled water), C = average percentage parasitaemia in the treated group.

Statistical analysis

The statistical analysis of this study was done using Statistical Package for Social Sciences (SPSS) version 21. One-way analysis of variance (ANOVA) was used to compare the means at a 95% confidence interval and the result was presented as mean standard deviation.

RESULTS AND DISCUSSION

Acute oral toxicity test (LD_{50}) for *D. tripetalla*

There were no signs of toxicity of *D. tripetalla* aqueous leaf extract in mice during the 96h observation period even at a dose of 2000mg/kg body weight, signifying that the leaf extract could be safely used at 2000mg/kg. Mordi *et al*., 2021 reported that there were no deaths observed within

the 48h period of administration of 2000 mg/Kg body weight of *D. tripetalla* which is indicative of the fact that the LD_{50} may be higher than the dose of 2000 mg/Kg body weight.

Curative action of *S. alata, D. tripetalla* and combined extracts of *S. alata* and *D. tripetalla*

The results of the curative action of *S. alata, D. tripetalla,* and combined extracts of *S. alata* and *D. tripetalla* are shown in Table 1. The highest parasitaemia level of 34.07±1.46% was reported on the first day of treatment in the negative control, this remained consistently high across the 5 days of treatment with percentages ranging from 34.07 to 38.50%. The lowest parasitaemia level was recorded in the reference drug chloroquine which ranged from 3.07±2.73% on day 1 to 0.07 ± 0.00% on day 5 with a percentage suppression range of 90.73% to 99.81% respectively. There were no parasites reported in the positive control (uninfected) group. Analysis of the five days treatment with the different concentrations of *S. alata*, when compared with the untreated group (negative control), showed a significant decrease ($p<0.05$) in parasiteamia level from day 1 to day 5 of treatment in a dose-dependent manner which ranged from 0.72 ± 0.0% on day 1 to 0.10 ± 0.12% on day 5 with a corresponding significant ($p<0.05$) suppression level of 98.13% (Day 5; 500 mg/kg) to 99.74% (Day 5; 2000mg/kg). This indicated that there was a steady decline in the percentage parasitaemia with an increase in treatment dose or concentration in each of the days. Similarly, analysis of the five days treatment with the different concentrations of *D. tripetalla* when compared with the untreated group (negative control) showed a significant decrease ($p<0.05$) in parasiteamia level from day 1 to day 5 of treatment in a dose-dependent manner ranging from 13.27±5.14% on day 1 to 8.40±2.03% on day 5 with a corresponding significant ($p<0.05$) suppression level of 96.88% (Day 5; 500 mg/kg) to 98.78 % (Day 5; 2000mg/kg). However, the combination of both *S. alata* and *D. tripetalla* exhibited a strong curative effect on malaria parasitaemia as shown in Table 1. There was a gradual significant clearance ($p<0.05$) in parasiteamia level from day 1 to day 5 of treatment in a dose-dependent manner which ranged from 12.94±0.03% to 1.09±0.02 % (500 mg/kg); 8.63±0.22% to 0.35±0.02% on day 1 to day 5 respectively, with a corresponding significant ($p<0.05$) suppression level of 97.17% (Day 5; 500 mg/kg) to 99.09% (Day 5; 2000mg/kg).

Prophylactic effect of treatment of *S. alata, D. tripetalla* and combination of both extracts on chloroquine-sensitive *Plasmodium berghei*

The prophylactic effect of the two test plants and their combined effect is shown in Figure 1. Aqueous extracts of S. *alata* and *D. tripetalla* exerted a significant decrease ($p<0.05$) in percentage parastaemia on chloroquine-sensitive *Plasmodium berghei* in a dose-dependent manner with S. *alata* exhibiting a higher prophylactic action on malaria parasitaemia across the different concentrations when compared to the negative control. Similarly, combined prophylactic action of aqueous extracts of S. *alata* and *D. tripetalla* exerted a significant decrease ($p<0.05$) on chloroquine-sensitive *Plasmodium berghei* in a dose-dependent manner when compared to the negative control.

Table 1: Curative potentials (% Parasitamia) of *S. alata, D. tripetalla* and the combined extracts of chloroquine-sensitive *Plasmodium berghei*

Groups	Day 1		Day 2		Day 3		Day 4		Day 5	
	% Par	% Sup	% Par	% Sup	% Par	% Sup	% Par	% Sup	% Par	% Sup
1	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100
2	34.07	0.00	35.53	0.00	36.4	0.00	38.1	0.00	38.5	0.00
3	3.07 ± 2.73^{c}	90.73	0.67 ± 0.64^{c}	98.03	0.60 ± 0.53^{c}	98.31	0.27 ± 0.12^{c}	99.26	0.07 ± 0.07^{c}	99.81
4	11.47 ± 2.57^{c}	66.33	8.07 ± 2.27^{be}	77.28	5.60 ± 0.31^{cd}	84.62	0.40 ± 0.12^{ce}	98.95	0.72 ± 0.07^{bi}	98.13
5	9.93 ± 2.03^{c}	70.85	5.80 ± 1.50^{bf}	83.68	3.67 ± 1.52^{bf}	89.92	0.27 ± 0.07^{bk}	99.29	0.14 ± 0.07^{j}	99.64
6	6.70 ± 1.80^{e}	80.33	5.47 ± 1.35^{bgi}	84.60	2.47 ± 1.97^{bgi}	93.21	0.20 ± 0.00^{df}	99.45	0.10 ± 0.12^{bh}	99.74
7	5.87 ± 2.76^{f}	82.77	3.93 ± 2.40^{bh}	88.94	2.47 ± 1.97^{ce}	93.21	0.20 ± 0.12^{df}	99.45	0.10 ± 0.12^{ce}	99.74
8	13.27 ± 5.14^{c}	59.91	10.53 ± 7.51^{c}	69.01	9.13 ± 1.79^{c}	74.3	1.13 ± 0.81^{c}	97.03	1.20 ± 0.87^{c}	96.88
9	11.73 ± 2.66^{c}	64.56	10.00 ± 1.44^{c}	70.65	7.00 ± 2.43^{c}	80.3	1.09 ± 0.31^{c}	97.13	0.60 ± 0.53^{c}	98.44
10	11.20 ± 7.48c	66.16	7.87 ± 2.50^{c}	79.25	6.40 ± 4.16^{c}	81.99	1.00 ± 0.72^{c}	97.38	0.55 ± 0.20^{c}	98.57
11	8.40 ± 2.03^{c}	74.62	6.53 ± 0.42^{c}	80.83	4.27 ± 1.85^{c}	87.98	0.73 ± 0.31^{c}	98.08	0.47 ± 0.31^{c}	98.78
12	12.94 ± 0.03^{g}	62.02	10.18 ± 0.02^{bj}	71.35	8.69 ± 0.06^{bhi}	76.13	1.17 ± 0.04^{df}	96.93	1.09 ± 0.02^{ce}	97.17
13	10.56 ± 0.06^{h}	69.00	8.32 ± 0.03^{bk}	76.58	7.79 ± 0.13^{bl}	78.60	0.98 ± 0.14^{bk}	97.42	0.89 ± 0.03^{de}	97.69
14	9.34 ± 0.10^{i}	72.59	7.41 ± 0.17^{bl}	79.14	5.19 ± 0.01^{bl}	85.74	0.78 ± 0.05^{dh}	97.95	0.62 ± 0.07^{bk}	98.39
15	8.63 ± 0.22^{j}	74.67	5.71 ± 0.02^{bk}	83.93	4.75 ± 0.04^{bk}	86.95	0.50 ± 0.02^{dj}	98.69	0.35 ± 0.02^{dh}	99.09

Values are expressed as mean ± standard error of mean (n=3). Values with similar superscripts letters along the column are not statistically significant at p>0.05. Treatment Group: 1 - Normal Control, 2 – Negative Control (Untreated), 3 – Chloroquine treated group, 4 - *S. alata* 500mg/kg, 5 - *S. alata* 1000mg/kg, 6 - *S. alata* 1500mg/kg, 7 - *S. alata* 2000mg/kg, 8 - *D. tripetalla* 500mg/kg, 9 - *D. tripetalla* 1000mg/kg, 10 - *D. tripetalla* 1500mg/kg, 11 - *D. tripetalla* 2000mg/kg, 12 - *S. alata + D. tripetalla* 500mg/kg, 13 - *S. alata + D. tripetalla* 1000mg/kg, 14 - *S. alata + D. tripetalla* 1500mg/kg, 15 - *S. alata + D. tripetalla* 2000mg/kg. %Par = Percentage Parasitaemia, %Sup = Percentage Suppression

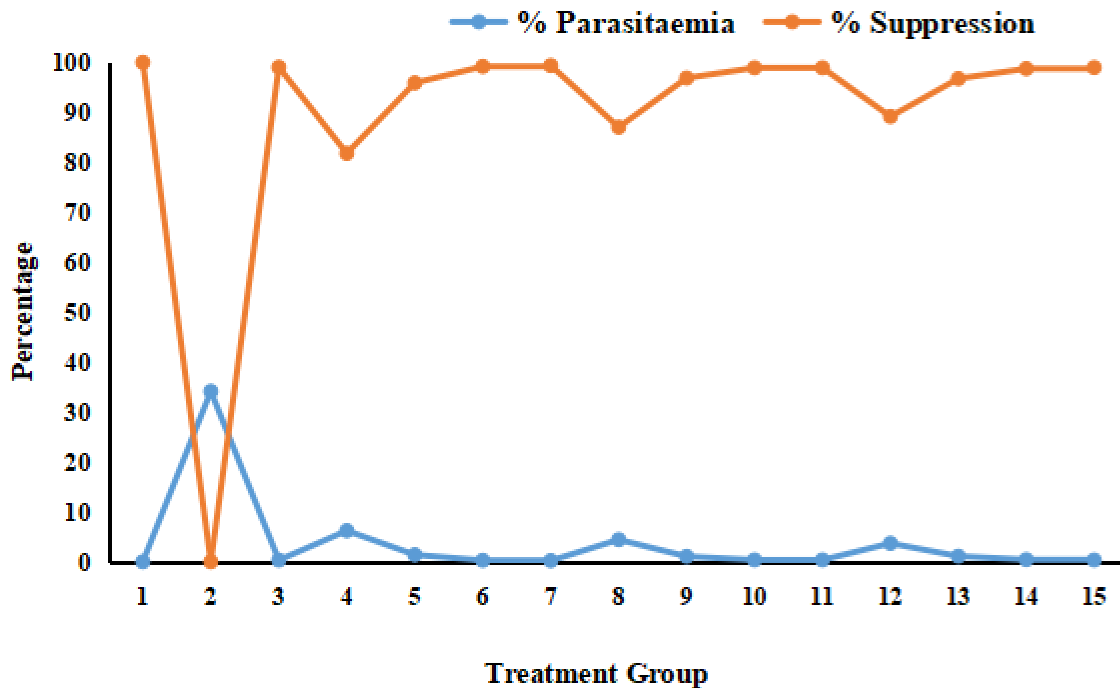

Figure 1 – Post 72 hours Prophylactic potentials of *S. alata, D. tripetalla* and the combined extracts on chloroquine-sensitive *Plasmodium berghei.* Treatment Group: 1 - Normal Control, 2 – Negative Control (Untreated), 3 – Chloroquine treated group, 4 - *S. alata* 500mg/kg, 5 - *S. alata* 1000mg/kg, 6 - *S. alata* 1500mg/kg, 7 - *S. alata* 2000mg/kg, 8 - *D. tripetalla* 500mg/kg, 9 - *D. tripetalla* 1000mg/kg, 10 - *D. tripetalla* 1500mg/kg, 11 - *D. tripetalla* 2000mg/kg, 12 - *S. alata* + *D. tripetalla* 500mg/kg, 13 - *S. alata* + *D. tripetalla* 1000mg/kg, 14 - *S. alata* + *D. tripetalla* 1500mg/kg, 15 - *S. alata* + *D. tripetalla* 2000mg/kg.

Chemo-suppressive effect of treatment of *S. alata, D. tripetalla,* and combination of both extracts on chloroquine-sensitive *Plasmodium berghei*.

The result of the study on chemosuppressive effect of treatment of *S. alata, D. tripetalla* and combination of both extracts on chloroquine-sensitive *Plasmodium berghei* indicated that treatment with *S. alata, D. tripetalla* and combination of both extracts exhibited a significant decrease ($p<0.05$) in percentage parastaemia on chloroquine-sensitive *Plasmodium berghei* in a dose-dependent manner Figure 2. The combination of both *S. alata* and *D. tripetalla* extracts at 2000 mg/kg exhibited a significant ($p<0.05$) chemo-suppressive parasitaemia of 1.39 % and suppression of 96.14% on malaria parasite used in the study. This is quite significant when compared to the negative control and the chloroquine-treated groups.

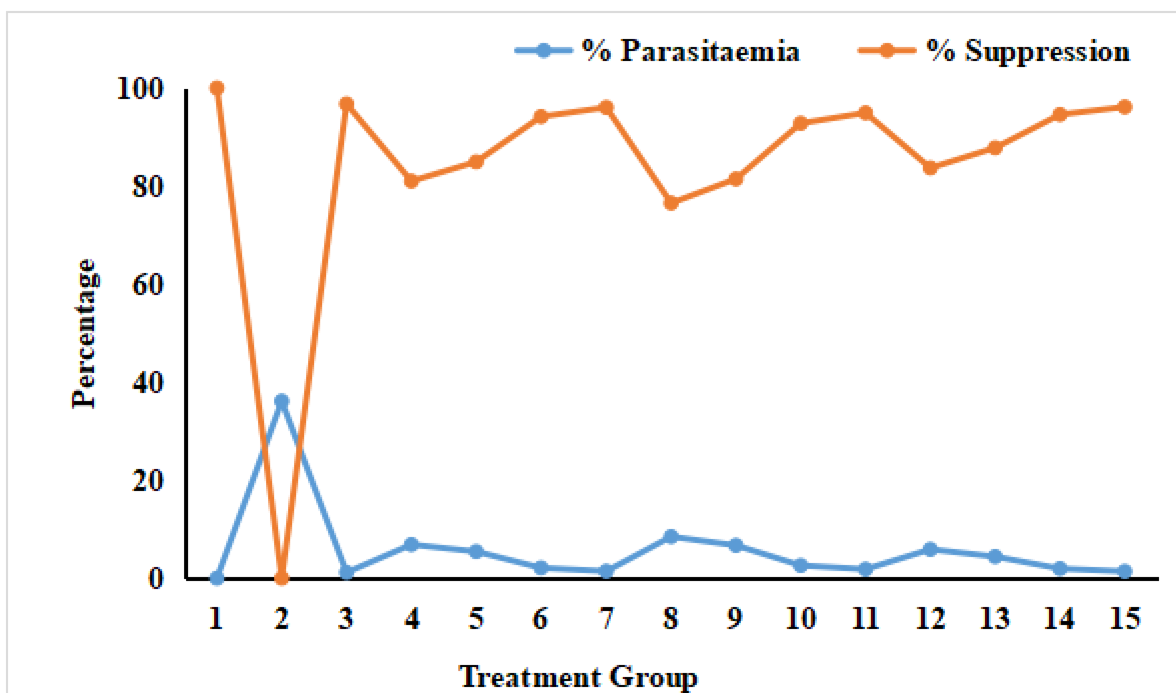

Figure 2 – Prophylactic potentials (%) of *S. alata, D. tripetalla* and the combined extracts on chloroquine-sensitive *Plasmodium berghei*. Treatment Group: 1 - Normal Control, 2 – Negative Control (Untreated), 3 – Chloroquine treated group, 4 - *S. alata* 500mg/kg, 5 - *S. alata* 1000mg/kg, 6 - *S. alata* 1500mg/kg, 7 - *S. alata* 2000mg/kg, 8 - *D. tripetalla* 500mg/kg, 9 - *D. tripetalla* 1000mg/kg, 10 - *D. tripetalla* 1500mg/kg, 11 - *D. tripetalla* 2000mg/kg, 12 - *S. alata* + *D. tripetalla* 500mg/kg, 13 - *S. alata* + *D. tripetalla* 1000mg/kg, 14 - *S. alata* + *D. tripetalla* 1500mg/kg, 15 - *S. alata* + *D. tripetalla* 2000mg/kg

The *P. berghei*-infected mouse model has been widely used as a preliminary test for the *in vivo* activity of potential antimalarial agents, as it provides a preclinical indication of any *in vivo* potential bioactivity as well as possible toxicity of the sample tested (Ang *et al*., 2000). Studies on phytochemical analyses of bioactive compounds present in the leaf extracts of *Senna alata* (L) *Roxb* and *D. tripetalla* showed the presence of saponins, phenolic acids, flavonoids, tannins (present as tannic acid), and alkaloids (Onyegeme-Okerenta, *et al.,* 2017a; Muhammed *et al.,* 2021). Similarly, dichloromethane extracts of *S. alata* and *D. tripetalla* leaves revealed the presence of secondary metabolites that have been shown to possess pharmacologic activities which may be responsible for the traditional use of these plants for the treatment of various illnesses (Onyegeme-Okerenta and Essien 2021).

The result of this study indicated a decrease in percentage parastaemia with an increase in dosage concentrations of the extracts. This simply reflects the higher potency of the extracts with a proportional increase in concentrations. Although the lethal concentration of *S. alata* which will pose a probable health hazard to the test animals was not reported in this study, however, Onyegeme-Okerenta, *et al*., (2017b) reported that 5000 mg/kg body weight of *S. alata* was the lethal dose (LD_{50}) that resulted in 50% mortality of test animals in an acute toxicity study previously carried out and from this study, there were no deaths observed within the 96 h period of administration of 500 - 2000 mg/Kg body weight of *D. tripetalla* which suggests that the LD_{50} may be higher than the dose of 2000 mg/Kg body weight. A study by White (2017) also reported similar findings, where it was documented that, higher doses correlated with higher effectiveness of the drug most especially at the first administration until the development of tolerance for the drug or plant extract.

Combination therapy of plants in ethnomedicinal studies and its diverse effects on test ailments have been reported (Adwan and Mhanna, 2009; Adwan *et al.*, 2009). This study reported an increase in the curative, chemo-suppressive, and prophylactic actions of the combination of both *S. alata,* and *D. tripetalla.* This result may be indicative of the fact that there are some levels of the synergistic effect of both plant combinations on malaria parasites. The result which also indicated that extract from both plants creates a better parasite clearance is also a pointer towards the potency of both plants as treatment plants rather than individual plants as reported by Alven and Aderibigbe (2019). It is important to note that Synergistic actions are not common in all extract combinations; there had also been counteractions of plant extract as documented by Zielinska-Blizniewska *et al*. (2019). Combinations of plants or drug extract should thus be extensively studied to determine their effectiveness either as synergistic or as counteractions as opined by Ogidi *et al*., (2021).

CONCLUSION

Aqueous extracts of *S. alata* and *D. tripetalla* have been reported to play crucial roles in the disease and general metabolisms of humans. This study, therefore, supported the traditional medicinal application of these plants and thus concludes that all the test plants (*S. alata, D. tripetalla,* and mixture of both) exhibited significant curative, chemo-suppressive, and concentrations of the extracts. It is worthy of note from the study that the combination of the two plant extracts yielded more effective curative, chemo-suppressive, and prophylactic measures on *P. berghei* infected mice in a dose-dependent manner. However, it is recommended that further fractionation and confirmatory analysis be carried out to elucidate and ascertain the secondary metabolites that would have resulted in the antiplasmodial activity of the plant extracts.

References

Adumanya OCU, Uwakwe AA, & Essien EB (2014). Antiplasmodial activity of methanol leaf extract of *Salacia senegalensis* Lam (Dc) in Albino Mice infected with chloroquine-sensitive *Plasmodium berghei berghei* (NK65). *International Journal of Ethnopharmacology,* 1(1):002-006.

Adwan G & Mhanna M (2009). Synergistic effects of plant extracts and antibiotics on *Staphylococcus aureus* strains isolated from clinical specimens. *Asian Pacific Journal of Tropical Medicine*, 2(3): 46-51.

Adwan G, Abu-shanab B, & Adwan K (2009). In vitro activity of certain drugs in combination with plant extracts against *Staphylococcus aureus* infections. *African journal of biotechnology*, 8(17).

Aliu YO & Nwude N (1982). *Veterinary Pharmacology and Toxicology*. 1st edition. pp: 104-110.

Alven S & Aderibigbe B (2019). Combination therapy strategies for the treatment of malaria. *Molecules*, 24(19): 3601.

Ang KKH, Holmes MJ, Higa T, Hamann MT, & Kara UAK (2000). "In vivo antimalarial activity of the beta-carboline alkaloid manzamine A," *Antimicrobial Agents and Chemotheraphy. 44*: 1645 – 1649.

Biu AA, Buratai LB, Konto M, Luka J, & Hauwa MM (2013). Acute toxicity study on aqueous extract of the leaf of *Cassia sieberiana* D.C. (Caesalpiniaceae) in albino rats. *An International Journal of the Nigerian Society for Experimental Biology*, pp 124–126

Braga CE, Martins AC, Cayotopa ADE, Klein WW, Schlosser AR, da Silva AF, de Souza NM, Andrade BWB, Filgueira-Junior JA, Pinto WDJ, & Nunes MDS (2015). Side Effects of Chloroquine

and Primaquine and Symptom Reduction in Malaria Endemic Area (Mancio Lima, Acre, Brazil). *Interdisciplinary Perspective on Infectious Disease*, 2015: 346853.

Calixto JB (2005). Twenty-five years of research on medicinal plants in Latin America: a personal view. *Journal of Ethnopharmacology*, 100(1-2):131-4.

Daniels RF, Deme AB, Gomis JF, Dieye B, Durfee K, & Thwing JI (2017). Evidence of non-*plasmodium falciparum* malaria infection in Kédougo Sénégal. *Malaria Journal*, 16 (1): 9.

Ehiowemwenguan G, Inetianbor JE, & Yakubu JM (2014). Antimicrobial Qualities of *Senna alata*. *Journal of Pharmacy and Biological Sciences*, pp 47-52

Ekor M (2013). The Growing Use of Herbal Medicines: Issues Relating To Adverse Reactions and Monitoring Safety. *Frontiers in Pharmacology*, 4:177.

Fairhurst RM, Gaurvika MLN, Breman JG, Hallett R, Vennerstrom LJ, Duong S, Ringwald P, Wellems ET, Plowe VC, & Dondorp MA (2012). Artemisinin-Resistant Malaria: Research Challenges, Opportunities, and Public Health Implications. *The American Journal of Tropical Medicine and Hygiene*, 87(2): 231–241

Federal Ministry of Health, Nigeria (FMOH). (2005). National Antimalarial treatment guidelines. Abuja, Nigeria, March. 5 - 22

Federal Ministry of Health Nigeria, (FMOH). (2011). National policy on malaria diagnosis and treatment. Abuja, Nigeria.

Fentahun S, Makonnen E, Awas T, & Giday M (2017). In vivo antimalarial activity of crude extracts and solvent fractions of leaves of *Strychnos mitis* in *Plasmodium berghei* infected mice. *BMC Complementary and Alternative Medicine*, 17 (1):13.

Hussain W, Lal Badshah L, Ullah M, Maroof MAA, & Hussain F (2018). Quantitative study of medicinal plants used by the communities residing in Koh-e-Safaid Range, Northern Pakistani-Afghan borders. *Journal of Ethnobiology and Ethnomedicine*, 14, 30.

Kayembe JS, Taba KM, Ntumba K, Tshiongo MTC, & Kazadi TK (2010). In vitro antimalarial activity of 20 quinones isolated from four plants used by traditional healers in the Democratic Republic of Congo. *Journal of Medicinal Plants Research*, 4(11), 991-994.

Lover AA, Dantzer E, Hongvanthong B, Chindavongsa K, Welty S, Reza T, Khim N, Menard D, & Bennett A (2018). Prevalence and risk factors for asymptomatic malaria and genotyping of glucose-6-phosphate (G6PD) deficiencies in a vivax-predominant setting, Lao PDR: implications for sub-national elimination goals. *Malaria Journal*, 17(1):218.

Mishra SK, Mohaptara S, Mohanty S, Patel NC, & Mohaptara DN (2002). Acute Renal Failure in *Falciparium Malaria*. *The Journal, Indian Academy of Clinical Medicine*, 3(2): 141-7.

Mordi JC, Ichipi-Ifukor PC, Kweki GR, Ichipi-Ifukor RN, Oyem JC, & Dennis-Eboh U (2021). Preliminary toxicology profile of *Dennettia tripetala* (Pepper Fruit) methanolic leaves extract. *Clinical Phytoscience* 7, 61.

Muhammed D, Adebiyi YH, Odey OB, Alawode AR, Lawal A, Okunlola MB, Ibrahim J, & Berinyuy BE (2021). *Dennettia tripetalla* (Pepper Fruit), a review of its ethnomedicinal use, phytoconstituents, and biological properties. *GSC Advanced Research and Reviews*, 06(03), 035-043.

Ochei J & Kolhatkar A (2000). Medical Laboratory Science: Theory and Practice. Tata McGraw-Hill, Page 966 – 967.

Ogidi CO, Ojo AE, Ajayi-Moses OB, Aladejana OM, Thonda OA, & Akinyele BJ (2021). Synergistic antifungal evaluation of over-the-counter antifungal creams with turmeric essential oil or *Aloe vera* gel against pathogenic fungi. *BMC Complementary Medicine and Therapies*, 21(1):1-12.

Onwujekwe O & Uzochukwu B (2005). Socio-economic and geographic differentials in costs and payment strategies for primary healthcare services in Southeast Nigeria. *Health Policy*. 71:383–97

Onyegeme-Okerenta BM, Nwosu T, & Wegwu MO (2017a). Proximate and phytochemical composition of leaf extract of *Senna alata* (L) Roxb. *Journal of Pharmacognosy and Phytochemistry,* 6(2): 320-326.

Onyegeme-Okerenta BM, Nwosu T, & Wegwu MO (2017b). Investigation of the Effect of Aqueous Leaf Extract of *Senna alata (L) Roxb* on Palm Oil-Induced Hyperlipidaemic Plasma Lipid in Wister Rats. *Clinical and Experimental Medical Sciences*, 5(1): 19 – 34.

Onyegeme-Okerenta BM & Essien EB (2021). Analysis of Bioactive Compounds Present in the Leaf Extracts of *Senna alata*, *Dennettia tripetalla* and *Delonix regia. Asian Journal Emerging. Research*, 3(1): 59-64.

Oputah IS, Oluseyi KA, Mordi RC, Olugbuyiro OAJ, Olorunshola J, & Azuh ED (2016). Phytochemical and Antibacterial Properties of Ethanolic Seed Extracts of Chrysophyllum albidum (African Star Apple). *Oriental Journal of Physical Sciences*, 1(1&2), 27-30.

Peters W & Robinson B (1992). The chemotherapy of rodent malaria. XLVII. Studies on pyronaridine and other Mannich base antimalarials. *Annals of Tropical Medicine and Parasitology*. 86(5):455–465. doi:10.1080/00034983.19 92.11812694

Pousibet-Puerto J, Salas-Coronas J, Sanchez-Crespo A, Molina-Arrebola MA, Soriano-Perez MJ, Gimenez-Lopez MJ, Vazquez-Villegas J, & Cabezas-Fernandez MT (2016). Impact of using artemisinin-based combination therapy (ACT) in the treatment of uncomplicated malaria from *Plasmodium falciparum* in a non-endemic zone. *Malaria Journal*, 15(1):339.

Schuster BG (2001). Demonstrating the validity of natural products as anti-infective drugs. *Journal of Alternate and Complementary Medicine*, 7: S73–S82.

Timothy SY, Lamu FW, Rhoda AS, Adati RG, Maspalma ID, & Askira M (2012). Acute toxicity, phytochemistry and antibacterial activity of aqueous and ethanolic leaf extracts of *Cassia alata* linn. *International Journal of Pharmacy*, **3**(6):2230-8407.

Ugbogu AE, Okezie E, Uche-Ikonne C, Duru M, & Atasie OC (2016). Toxicity Evaluation of the Aqueous Stem Extracts of *Senna alata* in Wistar Rats. *American Journal of Biomedical Research*, pp 80-86.

Wangdi K, Furuya-Kanamori L, & Clark J (2018). Comparative effectiveness of malaria prevention measures: a systematic review and network meta-analysis. *Parasite Vectors,* 11(1): 210.

White NJ (2017). Malaria parasite clearance. *Malaria journal*, 16(1), 1-14.

World Health Organisation. (2021). *World Malaria Report 2021*: *An in-depth update on global and regional malaria data and trends*. Global Malaria Programme, WHO. ISBN978-92-4-004049-6, https://www.who.int/teams/global-malaria-programme.

Zielinska-Blizniewska H, Sitarek P, Merecz-Sadowska A, Malinowska K, Zajdel K, Jablonska M, & Zajdel R (2019). Plant extracts and reactive oxygen species as two counteracting agents with anti- and pro-obesity properties. *International Journal of Molecular Sciences*, 20(18):4556.