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In vivo Antiplasmodial Activity of Raw Ethanolic Seed Extract of *Tetracarpidium conophorum* in Swiss Albino Mice Infected with *Plasmodium berghei*

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Authors' contributions

This work was carried out in collaboration between both authors. Author EOD designed the study, wrote the protocol and interpreted the data. Author OOO anchored the field study, gathered the initial data and performed the preliminary data analysis. Both authors managed the literature searches, produced the initial draft and approved the final manuscript.

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ABSTRACT

Study was carried out to determine the antiplasmodial activity of raw ethanolic seed extract of *Tetracarpidium conophorum*, in swiss albino mice infected with *Plasmodium berghei* (NK65). Standard methods were employed to determine the acute toxicity test, antiplasmodial activity and phytochemical screening of the seed extract. The experimental mice were acclimatized for seven days before the commencement of treatment. The mice were treated for four consecutive days with increasing dosages (200, 400, 600 mg/kg body weight) of seed extracts and a standard antimalarial drug (chloroquine as positive control) at a dose of 5 mg/kg body weight. Temperatures and body weights of mice were respectively measured each day. Qualitative phytochemical screening revealed the presence of phytate, oxalate, flavonoid, tannins, saponins, steroids, phenol, and terpenoids while quantitative screening revealed the presence of phytate (28.8 mg/g), oxalate

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(4.27 mg/100 g), tannins (0.59 mg/100 g), phenols (0.40 mg/100 g), alkaloids (0.31 mg/100 g) and flavonoids (0.21 mg/100 g) as secondary metabolites. The seed extract did not reveal any toxicity at all dosage levels used. The ethanolic seed extract revealed a dose-dependent activity in the chemosuppression of *Plasmodium berghei* by 2.77%, 30.55% and 47.22% at doses of 200, 400, 600 mg/kg body weight respectively while chloroquine at 5 mg/kg body weight produced 55.5% chemosuppression. Parasitemia in all the infected and treated experimental mice groups was significantly low ($P<0.05$) compared with the infected but untreated mice. The seed extract revealed a significant increase ($P<0.05$) in temperature and decrease in body weight of the infected mice. The study revealed that *Tetracarpidium conophorum* seed extract could be a future antiplasmodial herbal candidate for the treatment of malaria parasite.

Keywords: In vivo; Tetracarpidium conophorum; Antiplasmodial; Plasmodium berghei; albino mice.

1. INTRODUCTION

Malaria has been a severe and life threatening disease for thousands of years. The major impact of the disease is entirely on the developing countries, with the heaviest burden in Africa. It is one of the leading infectious diseases in many tropical regions, including Nigeria [1]. According to Bloland, [2], malaria is a complex disease whose epidemiology and clinical manifestations varies widely in different parts of the world as a result of the species of malaria parasite, their susceptibility to antimalarial drugs, distribution and efficiency of mosquito vectors, climate and level of immunity of the exposed human population. This parasitic disease is transmitted by the bite of an infected female *Anopheles* mosquito. Five *Plasmodium* species (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*) that infect human were recognized [3]. The fifth specie (*Plasmodium knowlesi*) is found throughout Southeast Asia as a natural pathogen of long-tailed and pig-tailed macaques has recently been implicated to be a major cause of human malaria in Malaysia [4]. *Plasmodium falciparum* is the most dominant, pathogenic and said to be responsible for almost all mortality caused by malaria in tropical and Sub tropical countries where the temperature and rainfall are optimum for the development of vectors and parasites [5]. In addition, four *Plasmodium* species (*Plasmodium berghei*, *Plasmodium chabaudi*, *Plasmodium vinckei* and *Plasmodium yoellii*) have been described in African murine rodents and occur geographically in Central Africa. *Plasmodium berghei* is transmitted by the bite of mosquito (*Anopheles dureni*). These parasites of rodents are practical model organisms in the laboratory for the study of human malaria aimed at the development of new vaccines and treatments [6].

According to WHO, [7], antimalarial drug resistance is a major health problem, which hinders the control of malaria. The use of chloroquine (CQ) to prevent and treat falciparum malaria has led to the widespread appearance of chloroquine resistant strains of *Plasmodium falciparum* throughout the world. It was opined by Krishna [8] that this resistance has increasingly extended to other available antimalarial drugs (amodiaquine, lumetantrine, mefloquine or sulfadoxine, pyrimethamine), consequently, the possible source for malaria treatment will appear to lie in the use of traditional herbal medicine. The use of plants for the treatment of malaria has extended to several countries in Africa, America and Asia [9].

Tetracarpidium conophorum (family Euphorbiaceae) seed is known as African walnut. In Nigeria, it is 'Ukpa' in Igbo and 'Awusa 'or Asala in Yoruba [10]. It is a west equatorial perennial plant often found growing in forests distributed in the southern part of Nigeria and West Africa. *Tetracarpidium conophorum* seed is highly medicinal and has been reported to be effective in the treatment of malaria [11]. The seeds are rich in flavonoid, tannins, alkaloid, protein, carbohydrate, fat and oils, vitamins and minerals which enhance its antimicrobial activities [12]. The aim of the study is to determine the *In vivo* antiplasmodial activity of the seed extract of the plant.

2. MATERIALS AND METHODS

2.1 Seeds Collection

The seeds of the plant *Tetracarpidium conophorum* were collected from a farmland in Akure, Ondo State. Seeds were identified and authenticated by Dr. Fayehun Lawrence, at the Department of Crop Soil and Pest (CSP)

Management, School of Agricultural Technology, The Federal University of Technology, Akure, Ondo State, Nigeria.

2.2 Preparation of Seed Extract

The seeds were separated from the hull, washed and dried at room temperature ($27\pm^{0}$C) for a month before grinding into a coarse powder with mortar and pestle then stored in an air tight bottle prior to analysis. Five hundred grams (500 g) of the grounded powder was soaked into 700 mls of 75% ethanol, stirred and left for 72 hours. The mixture was filtered using a millipore filter (pore size 0.7 um) and concentrated in a rotary evaporator at 40℃. The concentrate was heat dried over a water bath to obtain a solvent free extract and was refrigerated at 4℃.

2.3 Phytochemical Screening

Phytochemical analysis (qualitative and quantitative) was carried out according to standard procedures described by [13] to determine the bioactive constituents of ethanolic extract of *T. conophorum* seed.

2.4 Collection of Experimental Mice

Ethical use of experimental mice was in conformity with international, national and institutional guidelines for care and use of laboratory animals in Biomedical Research by Canadian council of animal care and United State National Institute of Health described by Adeneye, [14]. Male and female mice weighing between 15-22 g were obtained from animal house, Obafemi Awolowo University, Ile-Ife. Mice were housed in plastic cages with saw dust as beddings. They were fed with pellets (Supreme Pet food) and water ad libitum and acclimatized for 7 days at room temperature 29℃ -30℃ before the commencement of the experiment. A total of 24 mice were randomly divided into six groups (A (negative control), B (positive control), C (normal control), D, E and F) of four mice per group for the antimalarial activity and 16 mice were randomly grouped into four groups of four mice per group for acute toxicity test.

2.5 Grouping of Experimental Mice

Experimental mice were grouped according to the method of Berhan [15]. A total of 16 mice was used for acute toxicity test and were grouped into four groups (1 to 4) of four mice each, while a total of 24 mice were divided into six groups (A to F) of four mice per group for antimalarial activity.

2.6 Acute Toxicity

Acute toxicity test of the seed extract was carried out using [16]. Each mouse was respectively administered (400, 600, 900, 1000 mg/kg body weight) of *Tetracarpidium conophorum* ethanolic seed extract orally. The mice were observed daily for seven days for reduced activities, licking of paw, body weakness, sleeping and mortality

2.7 Measurement of Weight and Temperature of Mice

Body weights of the mice were measured throughout the study using a top pan animal balance. Temperatures of mice was measured using a BIOSEB (BIO9882) rectal thermometer with a probe inserted approximately 1.5 cm past the anal sphincter into the colon of hand-held mice. All the control groups and malaria – infected mice were observed visually daily throughout the experiment for behavioral changes which include diarrhea, lethargy, decreased activities, sleeplessness, loss of appetite.

2.8 Collection of Parasites

Chloroquine sensitive strain of malaria parasite (*Plasmodium berghei NK 65*) in a donor mouse was obtained from Obafemi Awolowo University, Ile-Ife Osun State, Nigeria. Parasite was kept alive by inoculating 0.2 mls of it into a healthy mouse (infected mouse) through intraperitoneal route. By cardiac puncture, 0.2 mls of the parasite was withdrawn from the infected mouse and serially diluted with sterile 4.8 mls of normal saline to obtain $1x10^{7}$ *Plasmodium berghei* infected erythrocyte. Mice in groups A, B, D, E and F were given 0.2 mls of the parasite after the parasitemia level of the infected mouse had been ascertained to be high. Group C (normal control) was not infected. Mice were visually observed for behavioral changes (decreased activities, loss of appetite) [17].

2.9 Determination of Parasitemia

Parasitemia in the mice was determined**,** using the method described by Hilou [18]. After 3 hours of infection with the *Plasmodium berghei*, the

seed extract was prepared by measuring 1.0 g, 2.0 g, 3.0 g on a sensitive scale and dissolving it into 5 ml of distilled water to produce concentrations of 200 mg/ml, 400 mg/ml and 600 mg/ml respectively and administered orally as treatment dose to mice in groups D, E, and F. Group B mice (positive control) received 5mg/kg of chloroquine, mice in group C (normal control) received 0.2 mls of normal saline, Mice in group A (negative control) were not treated. The treatment was administered for four consecutive days. On day five, parasitemia level of the mice (except group C) was determined by collecting a drop of blood on a microscope slide from each mouse by venesection of the tail. Thin blood smear was made and allowed to dry at room temperature. It was fixed with methanol before staining with 10% Giemsa for 10 minutes. The slides were allowed to air-dry, examined and counted under light microscope at X100 magnification (oil-immersion). The parasitemia was determined by counting minimum of three fields per slide with 100 RBC per field [19]. The percentage suppression of parasitaemia was calculated for each test concentration by comparing the parasitaemia in infected groups with those that received different concentrations of the test extract using [20]

Parasitemia = (Number of parasitized RBC x 100 / Total Number of RBC examined) (1)

Average % chemosuppression = (Parasitemia in negative control – Parasitemia in treatment x 100) / Parasitemia in negative control (2)

2.10 Data Analysis

Data were subjected to one way analysis of variance and was used to analyze data. $P< 0.05$ was considered significant difference between means (New Duncan's Multiple Range Test).

3. RESULTS

Secondary metabolites obtained from the qualitative screening (Table 1) revealed the presence of phytate, oxalate, flavonoid, tannins, saponins, steroids, phenol, terpenoids while quantitative screening (Fig. 1) revealed the presence of phytate (28.8 mg/g), oxalate (4.27 mg/100g), tannins (0.59 mg/100 g), phenols (0.40 mg/100 g), alkaloid (0.31 mg/100 g) and flavonoid (0.21 mg/100 g).

The result of acute toxicity is shown in Table 2. There were no behavioural signs of toxicity such as paw licking, sleeping, reduced activity, respiratory distress observed in mice and there was no mortality at all dosage levels used. The LD_{50} was therefore estimated to be greater than 1000 mg/kg body weight of the seed extract.

The effect of *Tetracarpidium conophorum* seed extract on body weight of mice is shown in Table 2. Significant reduction ($P< 0.05$) in weight was observed in mice in groups A, D, E and F respectively from pre-treatment to post-treatment periods. Group A (infected and untreated) reduced from 17.08 g to 14.17 g), group D (administered 200 mg/kg seed extract) reduced from 18.53 to 17.03g, group E (administered 400 mg/kg of seed extract) reduced from 20.33 to 16.70 g, group F (administered 600 mg/kg of seed extract) reduced from 22.40 to 17.86 g. There was no significant difference in the weight of the mice before infection and day one of infection except for the mice in group C. However, there was significant difference ($P< 0.05$) in the weights of the mice from day two to the last day of treatment. Mice in group C experienced increase in weight from 20.66 to 25.33 g.

The result obtained from Table 3 revealed decrease in the temperature of mice from 37 to 35.6℃ in group A as the number of day increases. Mice in the positive control (group B) also experienced reduction in body temperature from 36.5 to 34.5℃. There was a progressive increase in the body temperature observed in mice administered with 200 mg/kg, 400 mg/kg, and 600 mg/kg of the seed extract from 36.50 to 38.10℃, 36.8 to 38.2℃ and 36.5 to 37.6℃ respectively.

In-vivo antimalarial activity of seed extract (Fig. 2) show that at 200, 400, and 600 mg/kg concentration of the seed extract, mean parasite count of the mice was 34.9%, 24.85%, and 18.38% respectively. Chemosuppression value of the extract in groups D, E and F were 2.77%, 30.55%, and 47.22% respectively, while the value for chloroquine (group B) was 55.5%. Parasitemia in groups D, E, and F decreases with increase in dose levels of the extract. The observed value chemosuppression of mice administered 5 mg/kg body weight of chloroquine were significantly higher ($P< 0.05$) than mice in groups D, E and F. above were significantly higher ($P< 0.05$) compared with the mice in

group A. The parasitemia in the chloroquine treated mice is lower than mice administered with the highest dose of the seed extract.

4. DISCUSSION

Findings from this study revealed that the qualitative phytochemical screening of the ethanolic seed extract of *Tetracarpidium conophorum* contain secondary metabolites (alkaloids, oxalate, tannins, saponins, phenols, flavonoids, phytate).This is in agreement with the findings of Ajaiye oba and Fadare [10], Edem [12]. Quantitative phytochemical screening also revealed the presence of Phytate, Oxalate and Tannins which is in agreement with Enujiugha and Ayodele [21] who reported the presence of oxalates, phytates and tannins in raw *Tetracarpidium conophorum* seeds.

Observation on the acute toxicity test of the seed extract at different concentrations which revealed no mortality rate and behavioural signs of toxicity on all the experimental mice is expected. This indicates that the extract could probably be non-toxic. This is in agreement with Hodge and Sterner [22] who reported that based on Hodge and Sterner Toxicity Scale, any chemical exhibiting LD_{50} above 1000 mg/kg is practically non-toxic.

Decrease in mice weight observed on the second day of infection with the *Plasmodium* parasite, could presumably be due to the decrease in food intake and disturbed metabolic functions associated with malaria infection. This corroborates the findings of Basir [23]. The decrease in weight that was observed in mice treated with different concentration of the seed *extract* could be due to the presence of oxalate and tannins which has been reported to be antinutritional factors in *T. conophorum* Edem [12]. On the other hand, this decrease in mice weight could have been probably due the reason advanced that tannins reduce the bioavailability of proteins and protein value of foods Ford and Hewith [24]. Malaria, according to Basir [23] was reported to have induced low temperature in mice. This reason could have probably been responsible for the observed decrease in the temperature of the malaria infected experimental mice in this study. In addition, the decrease in temperature observed in the untreated *Plasmodium berghei* infected mice could be attributed to the debilitating effects of malaria on the host (mice) which could have brought loss of body heat due to the large surface area to body mass ratio of small animal like mice. This is in agreement with World Malaria Report [25]. Increase in temperature observed in mice administered with different concentrations (200 mg/kg, 400 mg/kg and 600 mg/kg) of *T.conophorum* seed extract agrees with findings of Ford and Hewith [24] who reported an increase in temperature of malaria infected mice treated with the extract of *Russelia equisetiformis*.

The *In vivo* screening of the seed extract for antiplasmodial efficacy which revealed decrease in parasitemia of groups D, E and F respectively compared to group A (negative control) is expected. The significant decrease in parasitemia observed was dose dependent which agrees with the finding of Ojo [26], on the antiplasmodial activity of *Rauvolfia vomitoria*. Alkaloids have been reported to have caused low parasitemia in Plasmodium infected mice Ajaiyeoba and Fadare [10]. The low parasitemia observed in this study could probably be due to presence of flavonoids, steroids and alkaloids which is also in line with the findings of Momoh [27] who working on the antiplasmodial activity of *Rauvolfia vomitoria* attributed low parasitemia in mice to the presence of flavonoid, steroids, and alkaloids. Low parasitemia observed in mice treated with chloroquine (group B) compared with mice treated with the seed extract is expected. This probably could be as a result of slow absorption of the seed extract by the mice, an observation which corroborate the findings of Ford and Hewith [24].

Table 1. Qualitative bioactive components of *Tetracarpidum conophorum* seed extract

Bioactive components	Inferences
Tannins	+
Flavonoid	+
Phytate	+
Alkaloids	+
Phenol	+
Oxalate	+
Terpenoids	+
Steroids	+
Saponoin	+
Phlobatannin	-

Key: + = Present and - = Absent

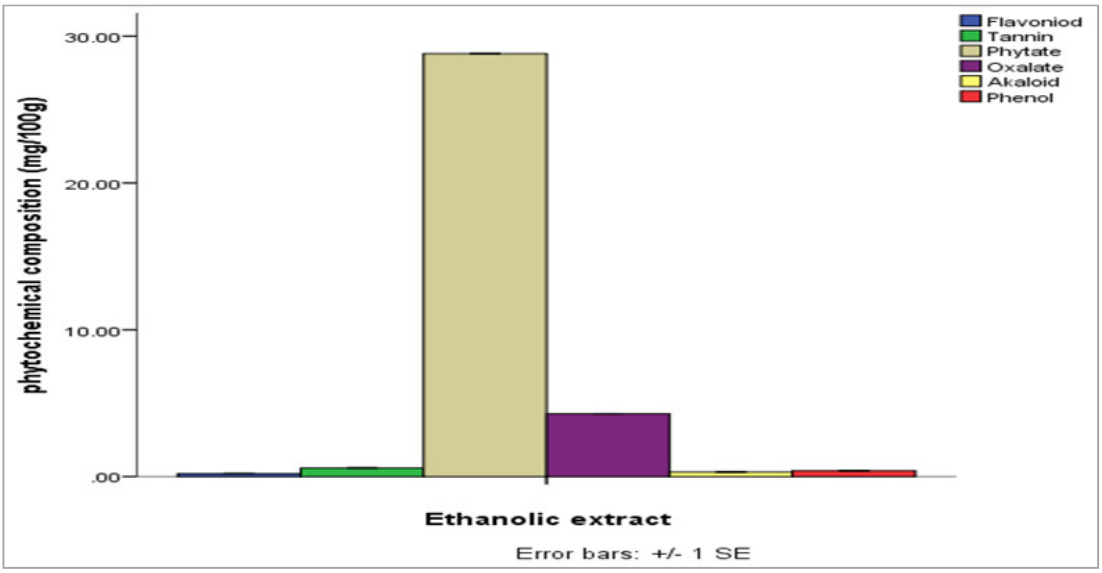

Fig. 1. Quantitative constittuents of the ethanolic seed extract of *Tetracarpidium conophorum*

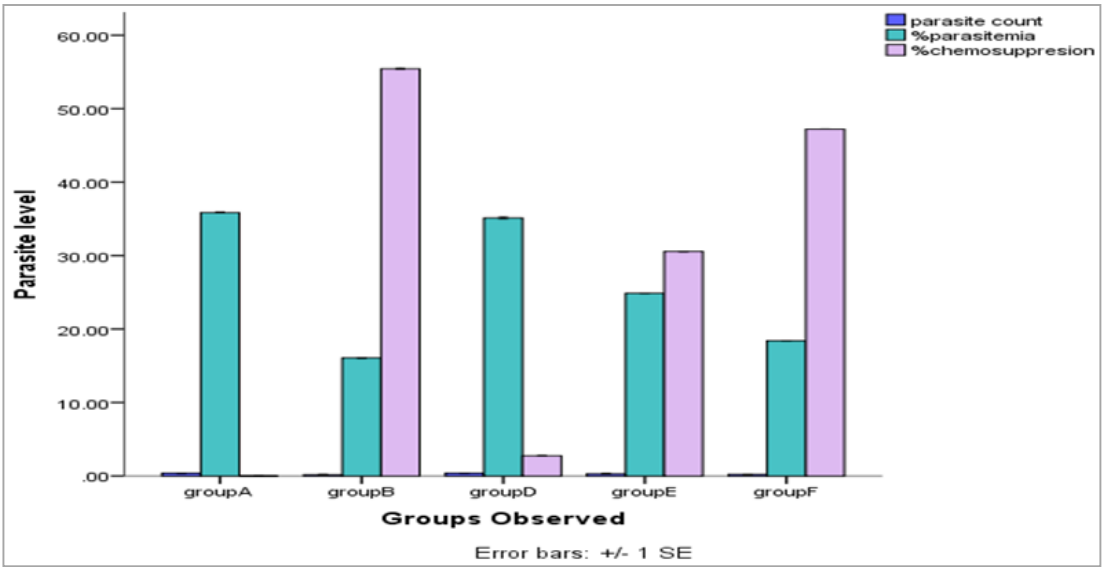

Fig. 2. Antiplasmodial activity of *Tetracarpidium conophorum* seed extract

Key: Group A = Infected and untreated, Group B = Infected and treated with Chloroquine, Group C = Not infected and not treated, Group D = 200 mg/kg of seed extract, Group E = 400 mg/kg of seed extract, Group F=600 mg/kg of seed extract.

Table 2a. Acute toxicity test

Groups	Dosage (mg/kg)	Mortality	Mortality	Signs of Toxicity
Group 1	400	0/4	0	Nil
Group 2	600	0/4	0	Nil
Group 3	900	0/4	0	Nil
Group 4	1000	0/4	0	Nil

Table 2b. Effect of *Tetracarpidium conophorum* on body weight of mice

Days	Weight of Mice (g)					
	Group A	Group B	Group C	Group D	Group E	Group F
Before	17.08±0.06^{d}	15.04±0.03^{a}	20.66±0.33^{a}	18.53±0.29^{b}	20.33±0.33^{c}	22.40±0.40^{c}
0	17.02±0.02^{d}	15.11±0.06^{a}	22.33±0.33^{b}	17.57±0.29^{b}	18.33±0.33^{b}	21.43±0.29^{c}
1	16.02±0.01^{c}	18.02±0.01^{b}	23.33±0.33^{b}	17.26±0.15^{a}	17.56±0.29^{b}	19.33±0.20^{b}
2	15.02±0.01^{b}	20.10±0.05^{c}	24.33±0.33^{cd}	16.33±0.33^{a}	17.30±0.15^{a}	18.26±0.15^{a}
3	14.17±0.12^{a}	20.14±0.07^{c}	25.33±0.33^{d}	17.03±0.61^{a}	16.70±0.17^{a}	17.86±0.46^{a}

Key: Group A = Infected and untreated, Group B = Infected and treated with Chloroquine, Group C = Not infected and not treated, Group D = 200 mg/kg of seed extract, Group E = 400 mg/kg of seed extract, Group F=600 mg/kg of seed extract.

Table 3. Effect of *Tetracarpidium conophorum* on body temperature of mice

	Temperatures of Mice (^{0}C)					
Days	Group A	Group B	Group C	Group D	Group E	Group F
Before	37.00±0.05^{cd}	36.50±0.25^{c}	36.93±0.28^{b}	36.50±0.15^{a}	36.83±0.12^{a}	36.56±0.09^{a}
0	37.27±0.12^{d}	35.67±0.44^{bc}	36.70±0.05^{c}	37.10±0.15^{b}	37.03±0.09^{ab}	36.83±0.09^{a}
1	36.57±0.18^{bc}	35.53±0.26^{abc}	36.10±0.15^{a}	37.03±0.20^{b}	37.27±0.09^{bc}	36.80±0.15^{a}
2	36.43±0.18^{b}	34.63±0.32^{ab}	36.03±0.23^{a}	37.10±0.06^{b}	37.40±0.06^{c}	37.43±0.12^{b}
3	35.63±0.20^{a}	34.53±0.29^{a}	36.02±0.38^{a}	38.13±0.08^{c}	38.20±0.06^{d}	37.60±0.10^{b}

5. CONCLUSION

The ever increasing global spread of drug resistance to the available antimalarial drugs is a major concern and requires innovative strategies to control the disease. This study has revealed the antiplasmodial efficacy of *Tetracarpidium conophorum* seed. The presence of alkaloids in the seed extract enhances its antiplasmodial activity. The plant is readily available in Nigeria to reduce economy cost. In view of this, the seed extract could be a folklore candidate in herbal medicine to be employed for the treatment of malaria. Furthermore, evaluation needs to be carried out before it could be employed for treatment of malaria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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