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ORIGINAL ARTICLE

Antiplasmodial activity of two medicinal plants against clinical isolates of *Plasmodium falciparum* and *Plasmodium berghei* infected mice

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Abstract Malaria is an infectious and deadly parasitic disease, associated with fever, anaemia and other ailments. Unfortunately the upsurge of plasmodium multidrug resistant constrained researchers to look for new effective drugs. Medicinal plants seem to be an unquenchable source of bioactive principles in the treatment of various diseases. The aim of this study was to assess the antiplasmodial activity of two Ivorian medicinal plants. The in vitro activity was evaluated against clinical isolates and *Plasmodium falciparum* K1 multidrug resistant strain using the fluorescence based SYBR green I assay. The in vivo bioassay was carried out using the classical 4 day suppressive and curative tests on *Plasmodium berghei* infected mice. Results showed that the in vitro bioassay of both plant extracts were found to exhibit a promising and moderate antiparasitic effects on clinical isolates (5 µg/mL $< IC_{50} <$ 15 µg/mL) and *Plasmodium falciparum* multidrug resistant K1 strain (15 µg/mL $< IC_{50} <$ 50 µg/mL). Furthermore, the in vivo antiplasmodial screening of both extracts showed a significant decrease in parasitemia, which was dose-dependent. Body temperature in mice treated with both extracts at experimental doses increased, compared to the negative control group and was dose-dependent. As for mice body weight a significant decrease ($p < 0.001$) was noticed in the negative control group compared to tested groups of animals. The hydroethanolic stem bark extract of *Anthocleista djalonensis* A Chev and leaves extract of *Ziziphus mauritiana* Lam exhibited antimalarial activities. Therefore, the bioactive compounds of both plant extracts need to be investigated.

Keywords *Anthocleista djalonensis* · *Ziziphus mauritiana* · Antiplasmodial activity · In vitro · In vivo

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Introduction

Malaria is an infectious and deadly parasitic disease caused by the genus plasmodium, transmitted to human beings through a bite of an infected mosquito of the anopheles genus and this disease is associated with fever, anemia and other ailments (Chen et al. 2016).

Many countries around the world were listed malaria risk area and according to the latest estimates from the World Health Organization there were 214 million new cases of malaria with a record of 438,000 malaria deaths globally (WHO, World malaria report 2016).

Furthermore, in areas with high transmission of malaria, children under 5 years of age are particularly susceptible to infection. In 2015, malaria killed about 303,000 including 292,000 in the African region (WHO, World malaria report 2016).

Indeed, malaria remains one of the world's most dreaded infectious parasitosis (Kaushik et al. 2013) and is still a major health problem in tropical countries (WHO, World malaria report 2015).

Artemisinin based combination therapies is the first line treatment against uncomplicated malaria on account of his highly effectiveness against *Plasmodium falciparum* (WHO, World malaria report 2015). Unfortunately, one of the major issue associated with malaria chemotherapy is drug resistance. Parasites are consistently been able to develop resistance to each new class of drugs, and the emergence of parasite resistant to artemisinin has been reported in Cambodia, Thailand, Myanmar, Laos and Vietnam, representing a great threat to effort to control and eventually eradicate malaria (Cui et al. 2015; WHO, Status report 2016). The problem is appalling and there is need for developing new drugs (Nondo et al. 2017).

For time immemorial medicinal plants have been a valuable source for the treatment of various diseases and an area to search for new antimalarials (Newman and Cragg 2016; Birru et al. 2017).

The objective of this study was to evaluate the antimalarial activity of the hydroethanolic stem bark extract of *Anthocleista djolonensis* A.Chev and leaves of *Ziziphus mauritiana* Lam two medicinal plants used by traditional healers.

Materials and methods

Collection and preparation of plant extract

Fresh leaves of *Ziziphus mauritiana* Lam and stem barks of *Anthocleista djalonensis* A.Chev were collected in Abidjan (Côte d'Ivoire). Both plants were authenticated at the Ivorian National Floristic Center (University of Felix Houphouet Boigny, Côte d'Ivoire). Then, plant samples were air dried in shade at room temperature and ground into powder. Then 100 g of each part of plant powder were separately macerated with 70% ethanol (1.5 L) at room temperature for 72 h and filtered through cotton sieve then on Whattman filter paper for 24 h. The filtrate was evaporated through rotary vacuum evaporator and dried in an oven at 45 °C for 48 h to obtain a dry extract which was stored at 4 °C (Zirihi et al. 2003) for further use.

Phytochemical screening test

The phytochemical screening of the hydroethanolic extracts of *Anthocleista djalonensis* A.Chev stem bark (HAd) and *Ziziphus Mauritiana* Lam leaves (HZm) were carried out to determine the presence of the following phytochemicals, Tanins, Alkaloids, Flavonoids, saponins, glycosides, sterols, terpenoids, quinones using standard procedures (Békro et al. 2007; Bidie et al. 2011).

In vitro antimalarial activity

Malaria parasites

Informed consent was obtained from all patients in this study prior to clinical isolates collection. Study approval was issued from the Ivorian National Ethical Committee and Research. Four fresh clinical isolates of *Plasmodium falciparum such as* ANKTC023, ANKTC024, ANKTC025 and ANKTC026 were obtained from symptomatic patients, at the Community Health Center of Anoukoua-Koute in the district of Abobo (Abidjan, Côte d'Ivoire). Moreover, *Plasmodium falciparum* multidrug resistant K1 strain (ATCC MRA-159, MR4, ATCC®Manassas, Virginia), obtained from USA was used for this study. The parasite was cultivated and maintained continuously in a human type O positive erythrocytes according to the method described by Trager and Jensen (1976).

In vitro antiplasmodial assay

Culture medium was consisted of RPMI 1640 medium [supplemented with 12.60 mL HEPES (25 mM), 100 mL hypoxanthine, 312.5 µL gentamycin (40 mg/mL) and glucose (20 g/L, Wagtech)]. Symptomatic blood samples of patients collected in EDTA collecting tubes were centrifuged at 3000 rpm for 5 min, then blood serum and buffy coat were removed and blood pellet washed thrice in RPMI 1640 medium (Gibco USA) and diluted with uninfected human type O positive red blood cells to reach a parasitemia of 0.24% at 1.5% hematocrit. Thawing of *Plasmodium falciparum* K1 strain was performed according to the method described by Witkowski et al. 2010, (2013). After withdrawing the cryovial from the nitrogen liquid, it was left thawing inside the Biosafety hood Class II (STERILGUARD) and transferred in a Falcon tube (15 mL) and then centrifuged at 3000 rpm for 5 min. The supernatant was removed, an equal volume of NaCl (3.5%) was added dropwise to blood pellet and slowly stirred. The tube was left resting for 1 min, then 12 mL of RPMI 1640 washing medium preheated at 37 °C was added and centrifuged at 3000 rpm for 5 min and the supernatant was removed. Then 50 µL of the blood pellet was suspended in 8 mL of complete medium in a culture flask cells (25 mL, Nunc WVR) and a volume of 110 µL of uninfected human type O positive red blood cells were added at 2% hematocrit. Daily, the infected blood pellets were transferred into fresh complete medium to propagate the culture.

The stock solution of both crude extracts and Chloroquine were dissolved separately, 10 mg of each substance

in 10 mL of distilled water to obtain a concentration of 1 mg/mL. Extract stock solutions were autoclaved at 121 °C for 15 min to sterilize them. As for Chloroquine a 0.22 µm Millipore filter was used for filtration. Aliquot of extracts and chloroquine were diluted in a complete medium and 100 µL of each aliquot was a twofold serial dilutions (100 µL) were performed in a 96 well microplate and concentrations ranged from 100 to 1.56 µg/mL for crude extracts and from 1600 to 3.125 nM for chloroquine. *Plasmodium falciparum* multidrug resistant K1 strain was synchronized by 10% D sorbitol (w/v) treatment at the ring stage prior to test. Then a volume of 100 µL of the inoculum (parasitized erythrocytes) was added to each well to reach a final volume of 200 µL. Infected erythrocytes non-treated with drugs were used as negative control whereas infected erythrocytes treated with chloroquine (CQ) were used as positive control. All experiments were run in duplicate. Microplates were confined in a candle jar saturated with CO_2 and incubated at 37 °C in an incubator for 72 h. After 72 h of incubation, microplates were preserved at − 20 °C.

Evaluation of parasitemia and determination of IC_{50}

After thawing of the 96 well microplates 100 µL of each well containing a volume of 200 µL was transferred in a new 96 well microplate and 100 µL of SYBR Green I lysis buffer (5 µL of SYBR Green was mixed to 25 mL of lysis buffer) was added to each well using a multi-channel pipette and incubated in a dark room at 37 °C for 1 h. Fluorescence was measured with a spectro-fluorimeter BIOTEK microplate reader (BIOTEK, FLX 800) with excitation and emission wavelength bands centered at 485 and 530 nm, respectively. IC_{50} (concentration of a tested substance inhibiting 50% of parasites growth) was determined through analysis of dose–response curves using the software IVART (In vitro Analysis and Reporting Tool) of WWARN (Le Nagard et al. 2011).

In vivo antimalarial bioassay

Animal material

Swiss albino mice of both sexes with body weight ranging from 22 to 25 g used for this study were obtained from the animal husbandry of the Department of Nutrition and Pharmacology, Faculty of Biosciences, University of Felix Houphouet Boigny (Abidjan, Côte d'Ivoire). Animals were housed in plastic cages in a temperature and light controlled room with 12 h dark and 12 h light cycle. They were fed with food pellets and given water ad libitum.

All experiments in this study were conducted in accordance with the international standards of animal welfare as recommended by the European Union legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU).

Parasite

The rodent malaria parasite *Plasmodium berghei* strain (chloroquine sensitive) ATCC-MRA-415 batch no 24,515,338 MR4 ATCC® Manassas, Virginia, was obtained from USA and stored in our Laboratory at − 196.4 °C and used for this study. *Plasmodium berghei* infected mice has been used because it induces a neurological syndrome in host known as experimental cerebral malaria, whose pathogenesis shares similarities with human cerebral malaria.

Parasite inoculation

Swiss mice previously infected by *Plasmodium berghei* - with a parasitemia rate of 23% were used as donors. The donor mice were anesthesia using ethyl ether and blood was collected through the jugular vein into heparinized vacutainer tube. The neck region of the animal was shaved and the jugular vein appears blue in color and a 25G needle was inserted in the caudocephalic direction (back to front) and blood was withdrawn slowly into EDTA vacutainer tube (Parasuraman et al. 2010). Blood was then diluted with physiological saline (0.9%) in such a way that 200 µL of blood contains 1×10^7 infected red blood cells. Each mouse was intraperitoneally given 200 µL of this diluted blood containing 1×10^7 *Plasmodium berghei* infected red blood cells.

Suppressive test

The chemo suppressive test of the hydroethanolic crude extracts of both plants was carried out according to the method described by Peters et al. 1975. Forty mice of both sexes were divided into 8 groups of 5 mice each. Mice of each group were intraperitoneally injected 200 µL of red blood cells containing 1×10^7 *Plasmodium berghei* infected erythrocytes. Three hours after mice infestation, all groups of animal were treated with extracts. Group 1 negative control (200 µL of distilled water); Group 2 positive control (5 mg/Kg of chloroquine); Group 3 (200 mg/Kg of Had), Group 4 (400 mg/Kg of Had); Group 5 (600 mg/Kg of Had); Group 6 (200 mg/Kg of HZm); Group 7 (400 mg/Kg of HZm); Group 8 (600 mg/Kg of HZm). Doses were administered daily by oral route for 4 consecutive days and crude extracts were dissolved in distilled water. On the fifth day (D_5), thin blood films were prepared from blood collected from mice tails and stained with 10% Giemsa (v/v).

Curative test

The curative potential of the hydroethanolic crude extracts were carried out according to the method described by Riley and Peters (1970). Forty mice of both sexes were divided into 8 groups of 5 mice each. On Day (0), healthy Swiss mice were intraperitoneally inoculated with 1×10^7 infected erythrocytes. Seventy-two hours later, mice were randomly distributed into their respective groups and were administered crude extract accordingly once daily for 5 days by oral route and crude extracts were dissolved in distilled water. Group 1 negative control (200 µL of distilled water); Group 2 positive control (5 mg/Kg of chloroquine); Group 3 (200 mg/Kg of Had), Group 4 (400 mg/Kg of Had); Group 5 (600 mg/Kg of Had); Group 6 (200 mg/Kg of HZm); Group 7 (400 mg/Kg of HZm); Group 8 (600 mg/Kg of HZm). Thin blood film made daily from the tail of each mouse 3 days after mice infection up to day 8 was stained with Geimsa and examined through a microscope (LEICA) with 100× magnification to monitor parasitemia level. Mean survival time for each group was calculated from date of infection over a period of 30 days (Chandel and Bagai 2010).

Monitoring of mice body weight and temperature

Body weight and rectal temperature of each mouse was measured before infection (day 0) and from day 3 up to day 8 after infection using a sensitive digital weighing scale and a digital thermometer respectively.

Parasitemia evaluation

Parasitemia was determined by counting on 12 fields of each slide to a total of 1000 red blood cells. For low parasitemia (< 1%) 30 fields about 4000 red blood cells were determined (Fidock et al. 2004). The percentage of parasitemia was calculated according to the following formula (Kalra et al. 2006; Toma et al. 2015).

$$\% \text{ Parasitemia} = (\text{Number of infected red blood cells} \times 100)/(\text{Total number of red blood cells})$$

Statistical analyses

Graphics were performed using Graphpad prism 5 software (Microsoft, San Diego California, USA). All values were expressed as mean ± Standard of deviation. Data analysis were performed using one way analysis of variance (ANOVA), followed by Tukey–Kramer multiple comparisms test using Graphpad instat® software. Values were statistically significant at $p < 0.05$.

Ethical consideration

This study was carried out according to the guidelines of the Ivorian National reference center for malaria chemoresistance created by the interministerial decree number 393/08/2006, and conduct research according to the Ivorian National Ethical Committee and Research. Therefore, this study was performed after receiving approval from the Ivorian National Ethical Committee and Research.

Results

Phytochemical screening

The hydroethanolic extraction of 100 g of the stem bark of *Anthocleista djalonensis* (HAd) or leaves of *Ziziphus mauritiana* Lam (HZm) gave a yield of 13.6 and 9.6% respectively. The phytochemical analysis results of both extracts showing the presence of some secondary metabolites are tabulated in Table 1. Results showed that the hydroethanolic extract of *Anthocleista djalonensis* contains terpenoids, steroids, cardiac glycosides, polyphenols and flavonoids. Whereas with *Ziziphus mauritiana* in addition to the secondary metabolites quoted above, the presence of coumarins, quinones, alkaloids and reducing sugar were noticed.

In vitro antiplasmodial activity

The hydroethanolic extracts of *Anthocleista djalonensis* stem bark and *Ziziphus mauritiana* leaves were tested on both clinical isolates and *Plasmodium falciparum* mul-

Table 1 Analysis of phytochemicals of hydroethanolic extracts of *Anthocleista djalonensis* stem bark and *Ziziphus mauritiana* leaves

Secondary metabolites	*Anthocleista djalonensis* A Chev	*Ziziphus mauritiana* Lam
Saponins	−	−
Terpenes and steroids	+	+
Alkaloids	−	+
Tanins	−	++++
Cardiac glycosides	+	++++
Flavonoids	+	++++
Coumarins	−	+
Quinones	−	++++
Polyphenols	+	++++
Reducing sugar	−	+++

++++, Highly abundent; +++, Very abundant; ++, Abundant; +, Present; −, Absent

Table 2 In vitro antiplasmodial activity of the hydroethanolic crude extract of *Anthocleista djalonensis* A Chev stem bark and *Ziziphus mauritiana* Lam leaves on clinical isolates and *Plasmodium falciparum* K1 strain

IC_{50} (μg/mL)					
Extract	Clinical isolates				*Plasmodium falciparum* strain
	ANKTC023	ANKTC024	ANKTC024	ANKTC024	K1
HAd	9.94	10	9.69	5.36	15.94
HZm	9.73	10.18	13.63	15.42	20
Chloroquine (CQ) (nM)	25.27	24.38	22.42	20.78	819.55

HAd, Hydroethanolic stem bark extract of *Anthocleista djalonensis* A Chev; HZm, Hydroethanolic leaves extract of *Ziziphus mauritiana* Lam

tidrug resistant K1 strain. The results of their antiplasmodial activity are recorded in Table 2. Both extracts showed a significant antiplasmodial activity on clinical isolates with IC_{50s} of 9.94; 10; 9.69 and 5.36 μg/mL for HAd and 9.73; 10.18; 13.63 and 15.42 μg/mL for HZm. HAd and HZm showed an outstanding antiplasmodial activity on clinical isolates. But, tested on *Plasmodium falciparum* multidrug resistant K1 strain, both extracts showed a moderate activity (15 μg/mL $< IC_{50} <$ 50 μg/mL).

In vivo antiplasmodial activity

Suppressive test

The hydroethanolic extracts of *Anthocleista djalonensis* A.Chev stem bark (HAd) and *Ziziphus mauritiana* Lam (HZm), reduced significantly ($p < 0.001$ in all cases) parasitemia in *Plasmodium berghei* infected Swiss mice at doses of 200; 400 and 600 mg/kg body weight, compared to negative control (Table 3), with inhibition percentage of 35.91; 47.86 and 70.55% for HAd and 58.68; 66.12 and 88.97% for HZm. The Inhibition (99.92%) obtained by chloroquine was significantly ($p < 0.001$) higher than both extracts notwithstanding the dose. Survival time increased and was statistically significant at doses of 400 and 600 mg/Kg body weight for both extracts (Table 3). As for the body weight, weight losses were recorded in each group of mice at day (5), Table 4.

Curative test

Parasitemia in the group treated with distilled water (negative control) increased steadily to reach 56.32% on day 8. As for the group treated with chloroquine (positive control group) parasitemia decreased from 15.75% on day 3 to 0% on day 8. On the other hand, groups treated with 200; 400 and 600 mg/Kg for both extracts exhibited suppression of parasite replication, entailing a reduction of their parasitemia compared to the negative control group (Fig. 1). Survival time increased and was statistically significant at doses of 400 and 600 mg/Kg bw for both extracts (Fig. 3).

As far as mice body weight was concerned the percentage of weight loss decreased for both extracts compared to the negative control group (Table 5). Moreover, the body temperature decreased steadily in the negative control group treated with distilled water. However, body temperature progressively increased in group of mice treated with chloroquine up to day 8. Eventually, a decrease of temperature was observed in groups of mice treated with extracts on day 4 followed by a progressive increase up to day 8, (Fig. 2).

As for mice survival time, it significantly increased according to dose of extracts, (Fig. 3).

Discussion

In this study, the antiplasmodial activity of two medicinal plant extracts was assessed. The in vitro test was evaluated on 4 clinical isolates and on *Plasmodium falciparum* multi drug resistant K1 strain. Whereas, the in vivo tests were assessed on *Plasmodium berghei* infected Swiss mice. According to the natural substance classification scale established by Bero et al. 2009, the hydrothanolic extracts of *Anthocleista djalonensis* A. Chev and *Ziziphus mauritiana* Lam showed a promising and moderate anti-malarial activity on both clinical isolates and *Plasmodium falciparum* multidrug resistant K1 strain respectively.

Thus, the antiplasmodial activity of both plant extracts in vitro was superior to that of *Ocimum sanctum* leaf extracts ($IC_{50} = 35.58$ μg/mL) and *Ocimum basilicum* ($IC_{50} = 43.81$ μg/mL) (Inbaneson and Sundaram, 2012). However, both plant extracts showed a low antiplasmodial activity compared to the dichloromethanic fraction of *Eremostachys macrophylla* rhizome ($IC_{50} = 0.797 \pm 0.016$ mg/mL) (Asnaashari et al. 2015).

The in vivo activity was ascertained using *Plasmodium berghei* infected mice required for antimalarial agents (Ang et al. 2001). The hydroethanolic stem bark extract of

Table 3 *In vivo* antiplasmodial activity of the hydroethanolic crude extract of *Anthocleista djalonensis* A Chev stem bark and *Ziziphus mauritiana* Lam leaves on *Plasmodium berghei* infected mice, 4 day suppressive test

Drug/extract	Dose (mg/Kg)	Mean ± SD parasitemia (%)	Suppression of parasitemia (%)	Mean survival time (days)
HAd	200	$16.04 \pm 1{,}94^{a,3}$	35.91	10.6 ± 1.4
	400	$13.05 \pm 1.42^{a,3}$	47.86	$13 \pm 1^{a,2}$
	600	$7.37 \pm 1.8^{a,3,b,3,c,3}$	70.55	$18.2 \pm 1.3^{a,3,b,2,c,1}$
HZm	200	$10.31 \pm 1.20^{a,3}$	58.68	$13.6 \pm 1.3^{a,1}$
	400	$8.48 \pm 1.42^{a,3}$	66.12	$17.2 \pm 1.64^{a,3}$
	600	$2.76 \pm 0.97^{a,3,b,3,c,3}$	88.97	$20.6 \pm 1.4^{a,3,b,2}$
Chloroquine	5	$0.019 \pm 0.001^{a,3,b,3,c,3}$	99.92	$30 \pm 0^{a,3,b,3,c,3}$
Distilled water	200 µL	25.03 ± 2.14	0	8 ± 1

Each value represents the mean ± standard deviation; (n = 5); values are statiscally different from control at $p < 0.05$; $p < 0.01$ and $p < 0.001$. One way analysis of variance (ANOVA) and Tukey–Kramer multiple comparisms test

HAd, Hydroethanolic stem bark extract of *Anthocleista djalonensis* A Chev; HZm, Hydroethanolic leaf extract of *Ziziphus mauritiana* Lam

[a]Compared to control

[b]To 200 mg/Kg

[c]To 400 mg/Kg

[d]To 600 mg/kg

[1]$p < 0.05$

[2]$p < 0.01$

[3]$p < 0.001$

Table 4 Effect of the hydroethanolic crude extract of *Anthocleista djalonensis* A Chev and *Ziziphus mauritiana* Lam on bodyweight of *Plasmodium berghei* infected mice in 4 day suppressive test

Groups	Dose	Mean body weight (D0)	Mean body weight (D5)	% Change
Negative control	200 µL	21.2 ± 0.4	19.17 ± 0.479	− 9.57
Chloroquine	5 mg/Kg	21.56 ± 0.2	21.35 ± 0.309	− 0.97
HAd	200 mg/Kg	20.88 ± 0.43	19.572 ± 0.380	− 6.27
	400 mg/Kg	21.09 ± 0.65	20.004 ± 0.193	− 5.16
	600 mg/Kg	21.1 ± 0.28	20.19 ± 0.27	− 4.31
HZm	200 mg/Kg	20.98 ± 0.43	19.87 ± 0.49	− 5.29
	400 mg/Kg	22.2 ± 0.27	21.12 ± 0.49	− 4.86
	600 mg/Kg	21.96 ± 0.28	21.46 ± 0.41	− 2.27

HAd, Hydroethanolic stem bark extract of *Anthocleista djalonensis* A Chev; HZm, Hydroethanolic leaf extract of *Ziziphus mauritiana* Lam

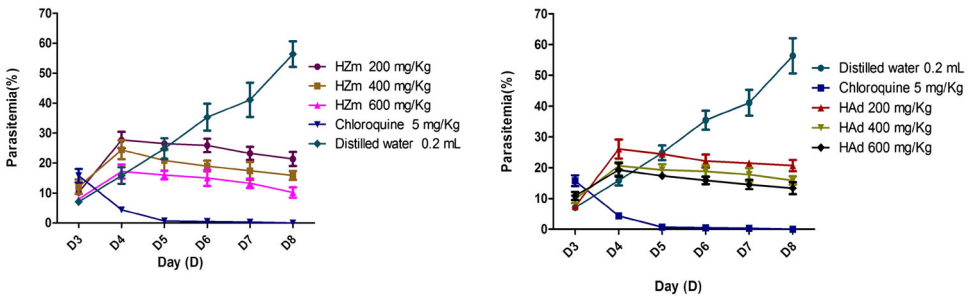

Fig. 1 Effect of the hydroethanolic crude extract of *Anthocleista djalonensis* A Chev and *Ziziphus mauritiana* Lam on *Plasmodium berghei* infected mice, Rane's test. HAd, Hydroethanolic stem bark extract of *Anthocleista djalonensis* A; HZm, Hydroethanolic leaf extract of *Ziziphus mauritiana* Lam

Table 5 Effect of the hydroethanolic crude extract of *Anthocleista djalonensis* A Chev and *Ziziphus mauritiana* Lam on bodyweight of *Plasmodium berghei* infected mice in curative test

Groups	Dose	Mean body weight (D0)	Mean body weight (D8)	% Change
Negative control	200 µL	22.52 ± 0.8	17.46 ± 0.7	− 22.46
Chloroquine	5 mg/Kg	22.47 ± 0.9	20.61 ± 0.5	− 8.27
HAd	200 mg/Kg	21.91 ± 0.2	18.22 ± 0.9	− 16.96
	400 mg/Kg	23.24 ± 0.7	20.81 ± 0.8	− 10.45
	600 mg/Kg	23.82 ± 0.4	20.34 ± 0.2	− 9.57
HZm	200 mg/Kg	25.5 ± 0.1	22.41 ± 0.3	− 12.11
	400 mg/Kg	24.92 ± 0.8	21.85 ± 0.4	− 11.61
	600 mg/Kg	25.5 ± 0.3	22.58 ± 0.6	− 9.2

HAd, Hydroethanolic stem bark extract of *Anthocleista djalonensis* A Chev; HZm, Hydroethanolic leaf extract of *Ziziphus mauritiana* Lam

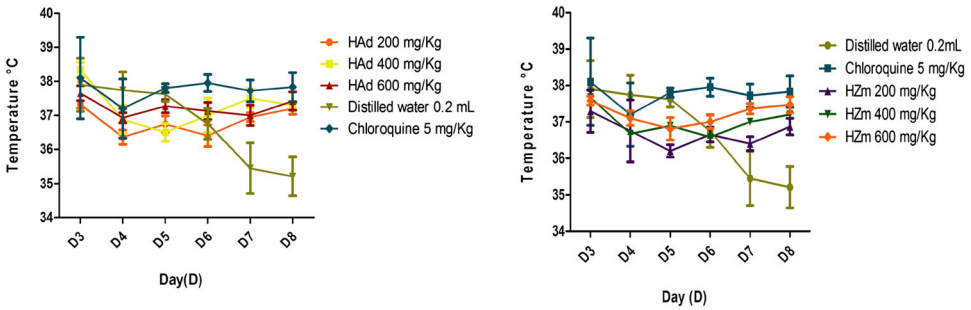

Fig. 2 Effect of the hydroethanolic crude extract of *Anthocleista djalonensis* A Chev and *Ziziphus mauritiana* Lam on body temperature of *Plasmodium berghei* infected mice in rane's test. HAd, Hydroethanolic stem bark extract of *Anthocleista djalonensis* A Chev; HZm, Hydroethanolic leaf extract of *Ziziphus mauritiana Lam*

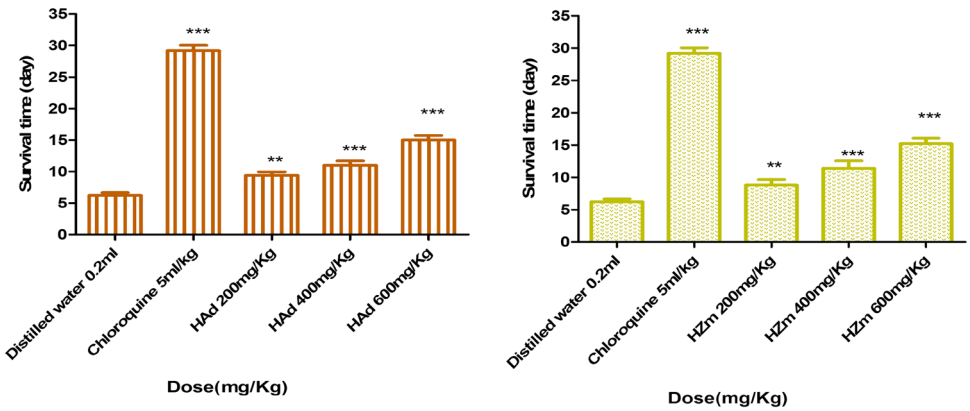

Fig. 3 Effect of the hydroethanolic crude extract of *Anthocleista djalonensis* A Chev and *Ziziphus mauritiana* Lam on the survival time of *Plasmodium berghei* infected mice in curative test. HAd, Hydroethanolic stem bark extract of *Anthocleista djalonensis* A Chev; HZm, Hydroethanolic leaf extract of *Ziziphus mauritiana Lam*

Anthocleista djalonensis A.Chev and leaves extracts of *Ziziphus mauritiana* Lam exhibited a good chemosuppression activity by reducing parasitemia to levels of 75.59 and 88.48% respectively at a dose of 600 mg/Kg in mice. The chemosuppression activity performed by HZm was not significantly different from that induced by chloroquine. As for the curative activity, HAd and HZm showed an increasing inhibitory activity at experimental doses. The phytochemical screening of both plant extracts revealed the presence of several phytocompounds such as alkaloids and terpenoids. Indeed, alkaloids are known for their antiplasmodial activity by blocking protein synthesis in *Plasmodium falciparum* (Atta-ur-Rahman and Choudhary, 1995; Mojarrab et al. 2014). Likewise, according to several pharmacological studies, terpenoids showed an outstanding activity against protozoans and malaria (Philipson and Wright 1991; Asase et al. 2010). Therefore, the antiplasmodial activity exhibited by both plant extracts could be

due to those phytocompounds which could have acted singularly or in association to produce the observed antiplasmodial effect. Furthermore, the promising activity could be due to the inhibition of parasite growth through fatty acid biosynthesis inhibition (Adams et al. 2005; Tasdemir et al. 2007). Plasmodium uses host hemoglobin as a nutrient for growth and multiplication. It ingests more than 75% of hemoglobin during its intra-erythrocytic phase and metabolizes heme into hemozoin (Inbaneson and Sundaram 2012). Thus, these extracts could have acted by blocking the formation of hemozoin in parasites. Moreover, the antiplasmodial activity observed with both plant extracts in vivo was comparable to that obtained with the ethyl acetate fraction of *Zehenria scabra* (Tesfaye and Alamneh 2014). On the other hand the extracts were more active than the methanolic, ethanolic and dichloromethanolic extracts of *Caesalpinia bonducella* (11.36, 20.01 and 37.60%) (Nondo et al. 2016).

In this study a significant weight loss was recorded in the control group treated with distilled water compared to groups of mice treated with chloroquine and crude extracts. The weight loss observed during experiment could be due to the lack of appetite causing a disturbed metabolic function and hypoglycemia (Basir et al. 2012).

In this study, the body temperature of mice decreases as parasitemia increases. The severe hypothermia observed in the negative control group treated with distilled water could be attributed to the general debilitating effect of malaria on host resulting in the loss of body heat and death (Basir et al. 2012).

But the progressive increase of body temperature in mice treated with extracts from day 5 might be due to a progressive decrease of parasitemia in treated mice and to the ability of extracts to prevent rapid falling of body temperature (Bantie et al. 2014). In this study mice treated at the dose of 600 mg/Kg with both extracts significantly lived longer than the negative control but was shorter as compared to chloroquine in the curative test. This longest survival time exhibited by both crude extracts might be due on one hand to the antiplasmodial activity of extracts and to the fast elimination phase of extracts from mice body (Muluye et al. 2015) and on the other hand to the presence of bioactive secondary metabolites in sufficient concentration at this dose (Fentahun et al. 2017).

Conclusion

The hydroethanolic stem bark extract of *Anthocleista djalonensis* A Chev and leaves extract of *Ziziphus mauritiana* Lam exhibited anti-malarial activities, therefore further studies of its bioactive compounds need to be investigated.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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