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Antiplasmodial and antipyretic studies on root extracts of *Anthocleista djalonensis* against *Plasmodium berghei*

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ABSTRACT

Objective: To evaluate the antimalarial activities of ethanolic root extract/fractions of *Anthocleista djalonensis* (*A. djalonensis*) in *Plasmodium berghei* (*P. berghei*) infected mice. **Methods:** *A. djalonensis* root extract (175-1 000 mg/kg) and fractions (chloroform, ethyl acetate and methanol; 250 and 500 mg/kg) were investigated for antiplasmodial activity against chloroquine-sensitive *Plasmodium berghei* infections in mice and for antipyretic activity against dinitrophenol, amphetamine and yeast-induced pyrexia. The antiplasmodial activity during early and established infections as well as prophylactic were investigated. Artesunate (5 mg/kg) and pyrimethamine (1.2 mg/kg) were used as positive controls. Antipyretic activity of the crude extract was also evaluated against dinitrophenol, amphetamine and yeast-induced pyrexia. **Results:** The extract and its fractions dose-dependently reduced parasitaemia induced by chloroquine sensitive *Plasmodium berghei* infection in prophylactic, suppressive and curative models in mice. These reductions were statistically significant ($P<0.001$). They also improved the mean survival time from 13 to 28 days relative to control ($P<0.001$). The activities of extract/fractions were comparable to that of the standard drugs used (chloroquine and pyrimethamine). On pyrexia induced by dinitrophenol, amphetamine and yeast, the extract inhibited significantly ($P<0.05$ - 0.001) and in a dose-dependent fashion temperature rise caused by these pyrogens. **Conclusions:** *A. djalonensis* root extract has antiplasmodial and antipyretic activities which may in part be mediated through the chemical constituents of the plant.

1. Introduction

Malaria has remained one of the most devastating diseases in tropical and subtropical regions of the world despite the global fight against the disease. The effect of malaria is greatly felt by the poor population of the third world countries such as those in Africa where the people cannot afford the costly effective drugs. The introduction of insecticide-treated nets, to some degree, has been helpful to the educated few who have appreciated its usefulness. Majority still do not make use of the nets perhaps due to cultural background and individual differences. Yet, the use of herbal remedies in the treatment of malaria has provided succor to this group of people and even to some enlightened ones in towns and villages. Investigations of antimalarial potentials of plants used in traditional therapy of malaria has been one of the means of searching for active antimalarial compounds that will liberate human race from the problem of malaria.

Anthocleista djalonensis (*A. djalonensis*) A. Chev (Loganiaceae) is a medium-sized tree of the West tropical Africa, 30-45 feet high with blunt spines on the unbranch, pale grey trunk and widespreading crown[1]. The stem, rootbark and leaves of *A. djalonensis* are used to treat malaria, jaundice, diabetes and abscesses[1]. The Ibibios of Southern Nigeria use the leaves and stembark as malarial remedy[2]. Onocha *et al* isolated monoterpene diol, djalonenol, as well as iridoid glucoside djalonenoside (also sweroside) and some of these compounds and their semisynthetic derivatives were found to be cytotoxic against the brain tumor transformed fibroblasts[3]. Reports on antibacterial and wound healing activities, *in vitro* anthelmintic activity and antiplasmodial activity have been published[2,4,5]. We investigated the antiplsmodial activity of ethanolic extract of the root and fractions to ascertain their

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ethnobotanical uses.

2. Materials and methods

2.1. Plant material

Roots of *A. djalonensis* (A.Chev) (Loganiaceae) were collected in August, 2010 from Nyan forest in Uruan area of Akwa Ibom State and authenticated by Dr. Margaret Bassey, a taxonomist in the department of Botany, University of Uyo, Uyo, Nigeria. A voucher specimen of the plant was deposited in the Faculty of Pharmacy Herbarium, University of Uyo, Uyo.

2.2. Plant extraction

The roots collected were washed with clean water and air-dried for 2 weeks. These dried roots were pulverized (reduced to coarse powder) using pestle and mortar. The powdered root sample (2.0 kg) was divided into two parts; one part was exhaustively macerated in ethanol for 72 h to allow for proper extraction (cold extraction), while the second part was successively and gradiently macerated for 72 h in each of these solvents, chloroform, ethyl acetate and methanol. The mixtures were filtered with filter paper. The liquid filtrate was concentrated and evaporated to dryness in vacuo at 40 ℃ using a rotary evaporator to obtain good yield. The yield of each extract was calculated and recorded. The dry extracts/fractions were stored in a refrigerator at 4 ℃ prior to use[6].

2.3. Phytochemical screening

Phytochemical screening of the crude extract was carried out employing standard procedures[7], to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, cardiac glycosides and others.

2.4. Animals

The animals (Swiss albino mice and rats) of both sexes were used for these experiments. They were obtained from University of Uyo Animal House. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea Feed) and water *ad libitum*.

2.5. Determination of median lethal dose (LD_{50})

The median lethal dose (LD_{50}) of the extract was determined using albino mice. The extract was administered intraperitoneally (*i.p.*) and the method of Miller and Tainter was adopted[8]. This involved the administration of different doses of the extract (100-1 000 mg/kg) to groups of six mice each. The animals were observed for physical manifestation of signs of toxicity. The number of deaths in each group within 24 h was recorded.

2.6. Microorganism

A chloroquine sensitive strain of *Plasmodium berghei* (*P. berghei*) (ANKA) was obtained from the National Institute of Medical Research (NIMER), Lagos and was maintained by subpassage in mice.

2.7. Parasite inoculation

Each mouse used in the experiment was inoculated with 0.2 mL of infected blood (*i.p.*) containing about 1×10^7 *P. berghei* parasitized erythrocytes. The inoculums consisted of 5×10^7 *P. berghei* erythrocytes per milliliter. This was prepared by determining both the percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations[9].

2.8. Drug administration

The drugs (artesunate and pyrimethamine), extract and fractions used in the antiplasmodial study were orally administered with the aid of a stainless metallic feeding cannula.

2.9. Evaluation of antiplasmodial activity of the extract/fractions

2.9.1. Evaluation of suppressive activity of the extract and fractions (4-day test)

This test was used to evaluate the schizonticidal activity of the extract, fractions and artesunate against early *P. berghei* infection in mice. This was done as described by Okokon and Nwafor[6]. Seventy two mice were randomly divided into 12 groups of six mice each. On the first day (D0), the mice were administered with the extract, fractions and artesunate. The mice groups 1-4 were administered with 175, 250, 500, and 1 000 mg/kg of crude extract, groups 5-7 were administered with the 250 mg/kg of the chloroform, ethyl acetate and methanol fractions respectively, while groups 8-10 were administered with 500 mg/kg of the chloroform, ethyl acetate and methanol fractions respectively. 5 mg/kg of artesunate was administered to group 11 (positive control), and 10 mL/kg of distilled water to group 12 (negative control). All the administrations continued for four consecutive days (D0 - D3) between 8 am and 9 am. On the fifth day (D4), thin blood film was made from tail blood. The film was then stained with leishman stain to reveal parasitized erythocytes out of 500 in a random field of the microscope. The average percentage suppression of parasitaemia was calculated in comparison with the controls as follows:

$$\frac{\text{Average \% parasitaemia in negative control} - \text{Average \% parasitaemia in positive groups}}{\text{Average \% parasitaemia in negative control}}$$

2.9.2. Evaluation of prophylactic or repository activities of extract and fractions

The repository activity of the extract, fractions and pyrimethamine (daraprim) was assessed by using the method described by Udobang *et al*[10]. The mice were randomly divided into 12 groups of six mice each. Groups 1–4 were administered with 175, 250, 500 and 1000 mg/kg/day of the extract respectively, while groups 5–7 were respectively given 250 mg/kg/day of the chloroform, ethyl acetate and methanol fractions, groups 8–10 were given 500 mg/kg chloroform, ethyl acetate and methanol fractions respectively. Group 11 was given 1.2 mg/kg/day of pyrimethamine (positive control) and group 12 was administered 10 mL/kg of distilled water (negative control). Administration of the extract/fraction/drug continued for three consecutive days (D0 – D2). On the fourth day (D3) the mice were inoculated with *P. berghei*. The parasitaemia level was assessed by blood smears seventy two hours later.

2.9.3. Evaluation of curative activities of extract and fractions (Rane's test)

This was used to evaluate the schizonticidal activity of the extract, fractions and artesunate in established infection. This was done as described by Udobang *et al*[10]. *P. berghei* was injected into another 72 mice (*i.p.*) on the first day (D0). Seventy two hours later (D3), the mice was divided randomly into twelve groups of six mice each. The mice group 1–4 were administered with the 175 mg/kg, 250 mg/kg, 500 mg/kg, 1000 mg/kg of crude extract respectively, groups 5–7 were administered with the 250 mg/kg of the chloroform, ethyl acetate and methanol fractions respectively, while groups 8–10 were administered with 500 mg/kg of the chloroform, ethyl acetate and methanol fractions respectively. 5 mg/kg of artesunate was administered to group 11 (positive control), and 10 mL/kg of distilled water to group 12 (negative control). The extract, fractions and drugs were administered once daily for 5 days. Leishman's stained thin smears were prepared from tail blood samples collected on each day of treatment to monitor parasitaemia level. The mean survival time (MST) of the mice in each treatment group was determined over a period of 29 days (D0 – D28).

$$\frac{\text{No. of days survived}}{\text{Total No. of days (29)}} \times 100 = \text{MST}$$

2.10. Evaluation of antipyretic activity of the extract

2.10.1. 2, 4–Dinitrophenol (DNP) induced pyrexia

Adult albino rats (160–186 g) of both sexes fasted for 24 hours but allowed water *ad libitum* were used for the experiment. They were randomized into groups of 6 rats each. DNP (10 mg/kg, *i.p.*) was administered to the rats after obtaining the basal rectal temperatures. Hyperthermia developed within 30 min of DNP administration. Different doses of extract (175, 250, 500 and 1000 mg/kg, *i.p.*), aspirin (100 mg/kg) and distilled water (10 mL/kg, orally) were administered respectively to the treatment and control groups of animals. Rectal temperatures of the animals were obtained at an hour interval for 5 h[11].

2.10.2. D–amphetamine induced pyrexia

Adult albino rats (148–172 g) of both sexes fasted for 24 h but allowed water *ad libitum* were used for the experiment. They were randomized into groups of 6 rats each. Amphetamine (5 mg/kg, *i.p.*) was administered to the animals after obtaining basal temperatures. Hyperthermia developed 0.5 h following amphetamine administration. The extract (175, 250, 500 and 1000 mg/kg *i.p.*), aspirin (100 mg/kg, orally) and distilled water (10mL/kg orally) were administered to the animals at peak hyperthermia. Rectal temperatures were obtained at 1 hour interval for 5 h[11,12].

2.10.3. Yeast induced pyrexia

Adult albino rats (140–180 g) of both sexes fasted for 24 h but allowed water *ad libitum* were used for the experiment. They were randomized into groups of 6 rats each. At zero hour, the basal temperature of the rats was taken using digital clinical thermometer. Thereafter, each animal was administered subcutaneously with 20% W/V aqueous suspension of yeast at a volume of 10 mL/kg. At suitable intervals beginning one hour after yeast injection, rectal temperature of animals were taken, animals with increase of 1 ℃ were selected and grouped for the study. The extract understudy was administered after the pyrogen at the dose of 175, 250, 500 and 1000 mg/kg (*i.p.*) to respective groups of rats. The control group received distilled water (10 mL/kg) and the reference group was administered with aspirin(100 mg/kg) both intraperitoneally. The rectal temperature of the groups was taken at 1 h interval for 5 h[11,12].

2.11. Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using Students' *t*–test and ANOVA (One– or Two–way) followed by a post test (Tukey–Kramer multiple comparison test). Differences between means will be considered significant at 1% and 5% level of significance *i.e.*, $P<0.01$ and 0.05.

3. Results

3.1. Acute toxicity

Administration of the ethanolic root extract of *A. djalonensis* (1000–5000mg/kg) after initial body weakness did

not produce any mortality in the animals. The median lethal dose (LD_{50}) was determined to be LD_{50} – 5000mg/kg.

3.2. Phytochemical screening

The *A. djalonensis* root extract was confirmed to contain flavonoids, saponins, tannins, cardiac glycosides and anthraquinones.

3.2. Antiplasmodial activity

3.2.1. Effect on suppressive activity of ethanolic root extract and fractions of A. djalonensis

The ethanolic root extract of *A. djalonensis* produced a dose dependent chemotherapeutic effect at the different doses employed in the study. The chemosuppressions were 37.66, 60.51, 67.92 and 76.55% for 175, 250, 500 and 1000 mg/kg/day doses respectively. The effects produced by the extract were statistically significant (P<0.001) relative to control. The effect of the highest dose (1000 mg/kg) exerted a chemosuppression (76.55%) comparable to that of standard drug (artesunate 5 mg/kg) with a chemosuppression of 74.07% (Table 1). The fractions exerted different levels of chemosuppression with ethyl acetate (500 mg/kg) exerting the highest effect with a chemosuppression of 87.66%. This was followed by chloroform and methanol with comparable chemosuppressions (64.81 and 61.11%) (Table 1).

Table 1
Suppressive activity of ethanolic root extract and fractions of *A. djalonensis* on *P. berghei* infection in mice (4-day test) (Mean ± SEM) (n=6).

Treatments	Dose (mg/kg)	Parasitaemia	% Chemosuppression
Normal saline	10 mL/kg	27.00 ± 2.02	–
A. djalonensis crude extract	175	16.83 ± 2.97**	37.66
	250	10.66 ± 2.44***	60.51
	500	8.66 ± 1.34***	67.92
	1000	6.33 ± 1.18***	76.55
Chloroform fraction	250	10.33 ± 1.56***	61.74
	500	9.50 ± 2.79***	64.81
Ethyl acetate fraction	250	11.83 ± 3.81***	56.18
	500	3.33 ± 1.18***	87.66
Methanol fraction	250	14.00 ± 2.61***	48.15
	500	10.50 ± 2.96***	61.11
Artesunate	5	7.00 ± 0.12***	74.07

**P<0.01, ***P<0.001 when compared with control.

Table 2
Repository/Prophylactic activity of ethanolic root extract and fractions of *A. djalonensis* on *P. berghei* infection in mice (Mean ± SEM) (n=6)

Treatments	Dose (mg/kg)	Parasitaemia	% Chemosuppression
Normal saline	10 mL/kg	14.33 ± 0.98	–
A.djalonensiscrude extract	175	9.16 ± 1.34***	36.07
	250	8.33 ± 1.69***	41.87
	500	4.66 ± 1.51***	67.48
	1000	2.83 ± 1.21***	80.25
Chloroform fraction	250	8.83 ± 2.76***	38.38
	500	6.16 ± 0.82***	57.01
Ethyl acetate fraction	250	3.16 ± 0.61***	77.94
	500	2.16 ± 0.86***	84.93
Methanol fraction	250	0.66 ± 0.36***	95.39
	500	0.50 ± 0.37***	96.51
Pyrimethamine	1.2	1.16 ± 0.71***	91.90

***P<0.001, when compared with control.

3.2.2. Prophylactic activity of ethanolic root extract and fractions of A. djalonensis

The ethanolic extract of *A. djalonensis* exerted a dose dependent prophylactic activity at the various doses employed resulting in significant (P<0.001) reduction of parasitaemia in extract treated groups when compared to control. Chemotherapeutic effects of 37.66, 60.51, 67.92 and 76.55% were respectively recorded for the corresponding dose of extract (175, 250, 500 and 1000 mg/kg/day). The chemosuppressions exerted by the middle and highest doses of the extract were comparable to that of the standard drug, pyrimethamine with chemosuppression of 78.96% (Table 2). The results of repository activities of the various *A. djalonensis* root fractions are shown in Table 2. Methanol fraction had the highest chemosuppression comparable

to that of the standard drug, artesunate 5 mg/kg. This was followed by ethyl acetate and chloroform.

Table 3
Mean survival time of mice receiving the various doses of ethanolic root extract and fractions of *A. djalonensis* during established *P. berghe*i infections in mice. (Mean ± SEM) (*n*=6)

Treatments	Dose (mg/kg)	MST (days)
Normal saline	10 mL/kg	11.33 ± 1.03
A. djalonensis crude extract	175	11.66 ± 0.22
	250	13.16 ± 0.93
	500	14.03 ± 0.48**
	1 000	15.83 ± 0.33***
Chloroform fraction	250	14.50 ± 0.54***
	500	15.33 ± 0.67***
Ethyl acetate fraction	250	14.33 ± 0.83b**
	500	14.83 ± 0.33***
Methanol fraction	250	11.66 ± 0.46
	500	13.66 ± 0.22*
Artesunate	5	14.83 ± 0.52**

$P<0.05$ **$P<0.01$, ***$P<0.001$, when compared with the control.

3.2.3. Curative activity of ethanolic root extract and fractions of A. djalonensis

The extract and its fractions showed a dose dependent schizonticidal effect on the parasitaemia similar to that of the artesunate treated group. These effects were statistically significant conpared to the control ($P<0.001$) (Figure 1, 2). The control group showed daily increase in parasitaemia.

The result of MST is shown in Table 3. The MST of the extract treated groups were significantly ($P<0.01$) longer than that of the control. Though both the extract and its fractions showed a significant dose dependent mean survival time on established infection ($P<0.001$), the crude extract, chloroform and ethyl acetate fractions showed greater protective effect with MST comparable to that of the standard drug, artesunate (5 mg/kg).

3.3. Antipyretic test

3.3.1. Dinitrophenol induced pyrexia

The antipyretic effect of the extract on DNP induced pyrexia is shown in Table 4. Administration of the root extract of *A. djalonensis* (175, 250, 500 and 1 000 mg/kg) in the presence of the pyrogen caused a significant ($P<0.05$ – 0.001) reduction in the temperatures of the extract treated rats when compared with the control. The antipyretic effect though non dose dependent was uncomparable to that of the

Table 4
Effect of *A. djalonensis* root extract on DNP induced pyrexia in rats. (Mean ± SEM) (*n*=6)

Treatment/dose (mg/kg)	Time intervals (h)						
	0	0.5	1	2	3	4	5
Control	37.05 ± 0.16	38.65 ± 0.13	38.90 ± 0.09	38.75 ± 0.08	38.38 ± 0.21	37.90 ± 0.18	37.78 ± 0.20
Extract 175	37.26 ± 0.10	38.15 ± 0.10	37.46 ± 0.08***	37.93 ± 0.07**	38.08 ± 0.05	37.60 ± 0.04	36.41 ± 0.06***
250	37.45 ± 0.13	38.88 ± 0.19	37.08 ± 0.43***	37.93 ± 0.17**	37.45 ± 0.16**	37.10 ± 0.16**	37.15 ± 0.17**
500	37.45 ± 0.19	38.18 ± 0.09	37.13 ± 0.12**	37.38 ± 0.10**	37.65 ± 0.10	37.50 ± 0.13	36.90 ± 0.14
1 000	37.06 ± 0.10	38.06 ± 0.10	37.65 ± 0.20**	37.98 ± 0.19**	37.66 ± 0.20	37.66 ± 0.20	37.31 ± 0.17*
aspirin 100	37.00 ± 0.12	38.86 ± 0.16	37.25 ± 0.10***	37.33 ± 0.15***	37.03 ± 0.23***	37.20 ± 0.11*	37.08 ± 0.19**

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ when compared with control.

Table 5
Effect of *A. djalonensis* root extract on amphetamine induced pyrexia in rat. (Mean ± SEM) (*n*=6)

Treatment/dose (mg/kg)	Time intervals (h)						
	0	0.5	1	2	3	4	5
Control	36.40 ± 0.08	37.50 ± 0.12	37.30 ± 0.13	37.30 ± 0.11	37.40 ± 0.10	37.60 ± 0.13	37.10 ± 0.11
Extract 175	35.70 ± 0.16	36.50 ± 0.10	36.40 ± 0.15*	35.30 ± 0.15***	37.00 ± 0.14	37.05 ± 0.17	37.20 ± 0.17
250	35.98 ± 0.23	36.68 ± 0.18	36.08 ± 0.32**	36.46 ± 0.32	37.05 ± 0.30	37.60 ± 0.31	36.91 ± 0.29
500	35.88 ± 0.22	37.13 ± 0.29	36.88 ± 0.18	37.10 ± 0.21	37.66 ± 0.20	36.08± 0.17***	37.26 ± 0.13
1 000	36.21 ± 0.25	37.00 ± 0.17	36.30 ± 0.17*	36.28± 0.19*	37.11 ± 0.20	37.09 ± 0.21	36.65 ± 0.22
aspirin 100	34.50 ±0.25	36.76 ± 0.17	36.50 ±0.17	36.16 ±0.19**	35.56 ± 0.20***	35.43 ± 0.21***	35.00 ± 0.22**

*$P < 0.05$, **$P <$ 0.01, ***$P < 0.001$ when compared to control.

Table 6
Effect of *A. djalonensis* root extract on yeast induced pyrexia in rat. (Mean ± SEM) (*n*=6)

Treatment/dose (mg/kg)	TIME INTERVALS (hr)						
	0	0.5	1	2	3	4	5
Control	38.82 ± 0.11	36.05 ± 0.15	38.17 ± 0.11	38.49 ± 0.08	38.90 ± 0.07	38.00 ± 0.06	38.29 ± 0.03
Extract 175	38.86 ± 0.15	38.31 ± 0.14	38.65 ± 0.11	37.66 ± 0.20*	37.70 ± 0.21***	37.78 ± 0.17	37.90 ± 0.16*
250	38.70 ± 0.12	38.51 ± 0.14	38.31 ± 0.12	38.17 ± 0.08	37.96 ± 0.09***	38.23 ± 0.08***	37.90 ± 0.09*
500	38.83 ± 0.09	38.35 ± 0.08	38.03 ± 0.06	38.00 ± 0.05	37.63 ± 0.05***	37.90 ± 0.08	37.90 ± 0.06*
1 000	38.65 ± 0.08	38.29 ± 0.11	37.98 ± 0.09*	37.86 ± 0.22	38.06 ± 0.06***	38.13 ± 0.06***	37.70 ± 0.06***
aspirin 100	38.03 ± 0.08	37.05 ± 0.11	36.56 ± 0.09***	36.20 ± 0.22***	35.86 ± 0.06***	35.46 ± 0.06***	35.22 ± 0.06***

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ when compared to control.

standard drug, aspirin (100 mg/kg).

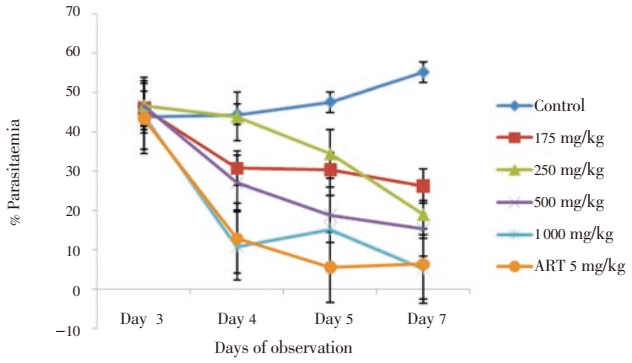

Figure 1. Curative effect of crude ethanolic root extrat of *A. djalonensis* on *P. berghei* established infection in mice. ART − Artesunate.

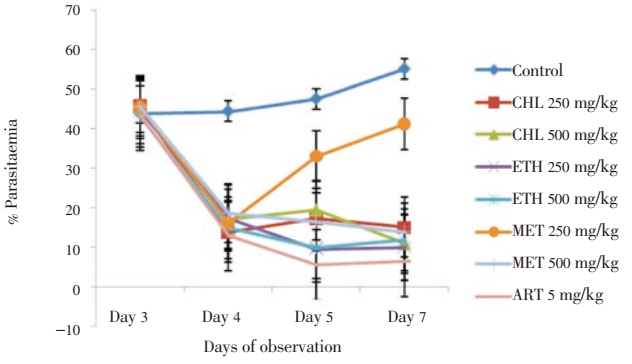

Figure 2. Curative effect of various root fractions of *A. djalonensis* on *P. berghei* established infection in mice. ART−Artesunate, CHL−Chloroform, ETH−Ethyl acetate, MET−Methanol.

3.3.2. Amphetamine induced pyrexia

Table 5 shows the effect of the extract on amphetamine induced pyrexia. The extract exerted a significant (P<0.05−0.001) non dose dependent antipyretic effect when compared to control. The antipyretic effect of the extract was uncomparable to that of the standard, aspirin (100 mg/kg).

3.3.3. Yeast induced pyrexia

The result of the effect of the extract against yeast−induced pyrexia is shown in Table 6. There was a progressive reduction in the temperature of rats treated with the extract. The reductions caused by the extract, though dose−dependent, was significant (P<0.05−0.001) when compared to control and comparable to that of the standard drug, aspirin (100 mg/kg) especially at the highest dose of the extract (1 000 mg/kg).

4. Discussion

Traditionally, the stem, rootbark and leaves of *A. djalonensis* have been reported to be use in the treatment of malaria, jaundice, diabetes and abscesses[1]. The Ibibios of Southern Nigeria use the leaves and stembark as malarial remedy[2]. The leaf and stembark have been confirmed to possess antiplasmodial activity[1].

These prompted the need to evaluate the *in vivo* antiplasmodial and antipyretic potentials of the crude extract and fractions of the root of *A. djalonensis* to confirm its antiplasmodial activity and ethnobotanical uses.

The antiplasmodial properties of the extract and its fractions were investigated using standard models. It was found that both the extract and its fractions significantly reduced the parasitaemia in suppressive, prophylactic and curative models in a dose dependent fashion. Some secondary metabolites of plants are said to have antiplasmodial activity. In addition, *A. djalonensis* has been reported to contain monoterpenes which have been implicated in antiplasmodial activities of plants[3]. Phytochemical compounds such as alkaloids and terpenes and their derivatives such as monoterpenes have been implicated in antiplasmodial activity of many plants[13]. Monoterpenes such as limonene have been implicated in endoperoxidation leading to plasmocidal activity[14]. These could have also contributed to the antiplasmodial activity of this extract and fractions.

On antipyretic activity, the extract inhibited significantly DNP, amphetamine and yeast induced pyrexia. DNP induces hyperthermia by uncoupling oxidative phosphorylation causing release of calcium from mitochondrial stores and also prevent calcium reuptake. This results in increased level of intracellular calcium, muscle contraction and hyperthermia[15]. Amphetamine acts on the brain causing the release of biogenic amines from their storage sites in nerve terminals resulting in increased level of cAMP and subsequent synthesis of prostaglandins from arachidonic acids produced in neurons by receptor−mediated hydrolysis of phospholipids[16]. This leads to hyperthermia. Yeast induces pyrexia by increasing the synthesis of prostaglandins[17-23]. The extract may in part reduced pyrexia by reducing brain concentration of prostaglandin E2 especially in the hypothalamus through its action on COX−2 or by enhancement of the production of the body's own antipyretic substances like vasopressin and arginine[24]. The hypothermic activity of the extract could have also been mediated by vasodilatation of superficial blood vessels leading to increased dissipation of heat following resetting of hypothalamic temperature control center[25-29]. This action may be due to the phytochemical compounds in this plant. Therefore, the temperature lowering activity of the extract may not be unconnected with the inhibition of one or combination of the mechanisms mentioned above.

The results of this study demonstrated that *A. djalonensis* possesses considerable antiplasmodial and antipyretic activities. These confirm its use to treat malaria and related symptoms in folkloric medicine. Therefore, it would be interesting if the the active principle is isolated, identified and characterised.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Dalziel JM. *The useful plants of West Tropical Africa*. London: Crown Agents for Overseas Colonies; 1954.

[2] Antia BS, Okokon JE, Etim EI, Umoh UF, Bassey EO. Evaluation of the *in vivo* antimalarial activity of ethanolic leaf and stembark extracts of *Anthocleista djalonensis*. *Indian J Pharmacol* 2009; **41**(6): 258-261.

[3] Onocha PA, Okorie DA, Connolly JD, Krebs HC, Meier B, Habermehl GG. Cytotoxic activity of the constituents of *Anthocleista djalonensis* and their derivatives. *Nig J Nat Prod Med* 2003; **7**: 58-60.

[4] Chah KF, Eze CA, Emuelosi CE, Esimone CO. Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants. *J Ethnopharmacol* 2006; **104**: 164-167.

[5] Nweze NE, Ngongeh LA. *In vitro* anthelmintic activity of *Anthocleista djalonensis*. *Nigerian Vet J* 2007; **28**(1): 9-13.

[6] Okokon JE, Nwafor PA. Antiplasmodial activity of ethanolic root extract and fractions of *Croton zambesicus*. *J Ethnopharmacol* 2009; **121**: 74-78.

[7] Trease GE, Evans WC. *Pharmacognosy*. 13th ed. London: Bailliere Tindall; 1989, p. 683-684.

[8] Miller LC, Tainter ML. Estimation of ED_{50} or LD_{50} values and their error using logarithmic-probit graph paper. *Proc Soc Exp Biol Med* 1944; **57**: 261-264.

[9] Okokon JE, Efiong IA, Etebong EO. Evaluation of *in vivo* antiplasmodial activities of ethanolic crude extract and fractions of leaf and root of *Carpolobia lutea*. *Pakistan J Pharmaceutical Sci* 2011; **24**(1): 57-61.

[10] Udobang JA, Nwafor PA, Okokon JE. Antiplasmodial activity of ethanolic and fractions of *Acalypha wilkensiana*. *J Ethnopharmacol* 2010; **127**: 373-378.

[11] Mbagwu HO, Anene RA, Adeyemi OO. Analgesic, antipyretic and antiinflammatory properties of *Mezoneuron benthamianum* Baill Caesalpiniaceae. *Nigerian Quarterly J Hospital Med* 2007; **17**(1): 35-41.

[12] Okokon JE, Nwafor PA. Antiinflammatory, analgesic and antipyretic activities of ethanolic root extract of *Croton zambesicus*. *Pakistan J Pharmaceutical Sci* 2010; **23**(4): 383-390.

[13] Christensen SB, Kharazmi A. Antimalarial natural products. Isolation, characterization and biological properties. In: *Bioactive compounds from natural sources: Isolation, characterization and biological properties*. Tringali C, editor. London: Taylor & Francis; 2001, p. 379-432.

[14] Hatzakis E, Opesenica I, Solaja BA, Stratakis M. Synthesis of novel polar derivatives of the antimalarial endoperoxides ascaridole and dihydroascaridole. *ARKIVOC* 2007; 8: 124 - 135.

[15] Kumar S, Baker K, Seger D. Dinitrophenol-induced hyperthermia resolving with dantrolene administration. *Clin Toxicol* 2002; **40**: 599-673.

[16] Westfall T, Westfall DP. Adrenergic agonists and antagonists. In: *Gilman and Goodman's the pharmacological basis of therapeutics*. 11th ed. New York: McGraw; 2006.

[17] Al-Ghamdi MS. The anti-inflammatory, analgesic and antipyretic activity of Nigella sativa. *J Ethnopharmacol* 2001; **76**: 45-48.

[18] SD Karou, T Tchacondo, L Ouattara, K Anani, A Savadogo, AAgbonon, et al. Antimicrobial, antiplasmodial, haemolytic and antioxidant activities of crude extracts from three selected Togolese medicinal plants. *Asian Pac J Trop Med* 2011; **4**(10): 808-813.

[19] Ajala TO, Igwilo CI, Oreagba IA, Odeku OA. The antiplasmodial effect of the extracts and formulated capsules of *Phyllanthus amarus* on *Plasmodium yoelii* infection in mice. *Asian Pac J Trop Med* 2011; **4**(4): 283-287.

[20] Okokon JE, Nwafor PA, Andrew UE. Antimalarial and analgesic activities of ethanolic leaf extract of *Panicum maximum*. *Asian Pac J Trop Med* 2011; **4**(6): 442-446.

[21] Attama AA, Igbonekwu CN. *In vitro* properties of surface-modified solid lipid microspheres containing an antimalarial drug: halofantrine.*Asian Pac J Trop Med* 2011; **4**(4): 253-258.

[22] Okokon JE, Etebong EO, Udobang JA, Obot J. Antiplasmodial and antiulcer activities of *Melanthera scadens*. *Asian Pac J Trop Biomed* 2012; **2**(1): 16-20.

[23] Samuel JI, Sundaram R. *In vitro* antiplasmodial activity of marine sponge *Hyattella intestinalis* associated bacteria against *Plasmodium falciparum*. *Asian Pac J Trop Biomed* 2012; **1**(Suppl 1): S100-S104.

[24] Chandrasekharan NV. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure and expression. *Proc Natl Acad Sci* 2002; **99**: 13926 - 13931.

[25] Rang HP, Dale MM, Ritter JM, Moore PK. *Pharmacology*. 6th ed. Edinburgh: Churchill Livingstone; 2007.

[26] Mohammad A, Mansoreh S, Mehdi K, Hasan V, Mohammad A. Persistence and residue activity of deltamethrin on indoor residual spraying surfaces against malaria vectors in southeastern Iran. *Asian Pac J Trop Biomed* 2011; **1**(Suppl 2): S271-S275.

[27] Zerihun T, Degarege A, Erko B. Association of ABO blood group and *Plasmodium falciparum* malaria in Dore Bafeno Area, Southern Ethiopia. *Asian Pac J Trop Biomed* 2011; **1**(4): 289-294.

[28] Krungkrai SR, Krungkrai J. Malaria parasite carbonic anhydrase: inhibition of aromatic/heterocyclic sulfonamides and its therapeutic potential. *Asian Pac J Trop Biomed* 2011; **1**(3): 233-242.

[29] Prabhu K, Murugan K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S. Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; **1**(2): 124-129.