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Antipyretic and antimalarial activities of crude leaf extract and fractions of *Enicostema littorale*

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ABSTRACT

Objective: To evaluate the antiplasmodial and antipyretic activities of whole plant extract and fractions of *Enicostemma littorale* (*E. littorale*) for ascertaining the folkloric claim of its antimalarial and antipyretic activities. **Methods:** The crude extract (260 – 780 mg/kg) and fractions (chloroform and acqeous; 520 mg/kg) of *E. littorale* were investigated for antiplasmodial activity against chloroquine-sensitive *Plasmodium berghei* (*P. 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 fractions dose-dependently reduced parasitaemia induced by chloroquine-sensitive *P. 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 11 to 27 days relative to control ($P<0.01 - 0.001$). The activities of extract/fractions were comparable to that of the standard drugs used (artesunate and pyrimethamine). On pyrexia induced by dinitrophenol, amphetamine and yeast, the extract caused inhibitions which were statistically significant ($P<0.05 - 0.001$) and in a dose-dependent fashion. **Conclusions:** These plant extracts possess considerable antiplasmodial and antipyretic activities, which justify its use in ethnomedicine.

1. Introduction

Malaria has been a devastating disease that affect populations living in most tropical and subtropical areas of the world where the disease is endemic. However, the use of natural products from plants have been very successful in the therapy of malaria. For example quinine from *Cinchona* species and artemisinin from *Artemisia annua* have been of immense help in the global fight against malaria. Plants have therefore been a dependable weapon in the fight against malaria for ages probably because the available effective antimalarial drugs are expensive, unaffordable and sometimes unavailable to these rural populations, while herbal remedies are readily available and inexpensive. These perhaps have been the reasons why some urban dwellers have also patronised herbal remedies in their treatment of malaria infections. 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 claws of malaria.

Enicostemma littorale (*E. littorale*) Blume syn *E. axillare* (Lam) Raynal (family – Gentianaceae) is a perennial herb common in the coastal areas of India and Tropical Africa. It is commonly known as chota-kirayata or chirayata (Hindi), mamejavo (Gujarat), and mfang idim (Ibibio). The plant is used in folk medicine to treat diabetes, rheumatism, abdominal ulcers, hernia, swelling, itching and insect poisoning[1]. Hot water extract of the plant is used in India to treat diabetes, fever, stomachache, dyspepsia and malaria[1]. Biological activities of the plant extracts such as antiinflammatory and antidiabetic[1-4], *in vitro* antioxidant and antimicrobial activities[5,6], anticancer[7] and *in vitro* antiplasmodial activity against *P. falciparum*[8] have been reported. We report in this study the *in vivo* antimalarial and

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antipyretic activities of *E. littorale* in *Plasmodium berghei (P. berghei)* infections in rodents.

2. Materials and methods

2.1. Plant materials

Fresh whole plants of *E. littorale* were collected from Ahembabad Gujrat, India and were identified and authenticated as *E. littorale* by Dr. R. C. Verma, School of Studies of Botany, Vikram University, Ujjain and a voucher specimen was deposited at Vikram University, Ujjain herbarium.

2.2. Extraction

Fresh whole plants were air-dried, pulverized using a pestle and mortar. The powdered whole plant (1 kg) was cold-macerated for 72 h using ethanol. The liquid ethanolic extract that was obtained by filtration was evaporated to dryness in a waterbath at 60 ℃ and part (10 g) of the crude extract was partitioned with a 50:50 mixture of distilled water and chloroform. The acqueous fraction was evaporated to dryness in a water bath at 60 ℃ and the chloroform fraction air-dried. The ethanolic cude extract, the acqueous and chloroform fractions were stored at −4 ℃ until used.

2.3. Animals

The animals (Swiss albino mice and rats) both male and female that were used for these experiments 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*. Permission and approval for animal studies were obtained from College of Health Sciences Animal Ethics committee, University of Uyo.

2.4. Microorganism

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

2.5. Determination of Median Lethal dose

The median lethal dose (LD_{50}) of the extract was estimated using albino mice by intraperitoneal route using the method of Tainter and Miller[9]. This involved intraperitoneal administration of different doses of the extract (100-1000 mg/kg) to groups of six mice each. The animals were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. The number of deaths in each group within 24 h was recorded.

2.6. Parasite inoculation

Each mouse used in the experiment was inoculated intraperitoneally with 0.2 mL of infected blood containing about 1×10^7 *P. berghei berghei* parasitized erythrocytes. The inoculum consisted of 5×10^7 *P. berghei berghei* erythrocytes per mL. 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[10].

2.7. 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.

Evaluation of antiplasmodial activity of the extract/fractions

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

This test was used to evaluate the schizontocidal activity of the extract, fractions and artesunate against early *P. berghei berghei* infection in mice. This was done as described by Okokon and Nwafor[10]. Forty-two mice were randomly divided into seven groups of six mice each. On the first day (DO), the forty-two mice were infected with the parasite and randomly divided into various groups. These were administered with the extract, fractions and artesunate. The mice group 1 were administered with the 260 mg/kg, the group 2 520 mg/kg and group 3 780 mg/kg of crude extract, groups 5 and 6 were administered with the 520 mg/kg of the acqueous and chloroform fractions respectively, while group 6 was administered with 5 mg/kg of artesunte (positive control), and 10 mL/kg of distilled water to group 7 (negative control) 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's 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:

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

2.8. Evaluation of prophylactic activities of extract and fractions

The repository activity of the extract, fractions and pyrimethamine (daraprim) was assessed by using the method described by Okokon and Nwafor[10]. The mice were randomly divided into seven groups of six mice each. Groups 1 − 3 were administered with 260, 520 and 780 mg/kg/day of the extract respectively, while group 4 − 7 were respectively given 520 mg/kg/day of the acqueous and chloroform fractions, 1.2 mg/kg/day of pyrimethamine (positive control) and 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 72 h later.

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

This was used to evaluate the schizontocidal activity of the extract, fractions and artesunate in established infection. This was done as described by Okokon and Nwafor[10]. *P. berghei berghei* was injected intraperitoneally into another 42 mice on the first day (D0). Seventy-two hours later (D3), the mice was divided randomly into seven groups of six mice each. Different doses of the extract, 260 mg/kg, 520 mg/kg and 780 mg/kg were orally administered respectively to mice in groups 1-3. 520 mg/kg of the acqueous and chloroform fractions were administered to groups 4 and 5 respectively, 5 mg/kg/day of artesuante to the group 6 (positive control) and group 7 was given 10 mL/kg of distilled water (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 (150 - 170 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 (260, 520 and 780 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 (150 - 170 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 (260, 520 and 780 mg/kg, *i.p.*) aspirin (100 mg/kg orally) and distilled water (10 mL/kg orally) were administered to the animals at peak hyperthermia. Rectal temperatures were obtained at 1 h interval for 5[11].

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[11]. 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 *i.p.* after the pyrogen at the dose of 260, 520 and 780 mg/kg to respective groups of rats. The control group received distilled water (10 mL/kg) and the reference group administered with ASA (100 mg/kg) both intraperitoneally. The rectal temperature of the groups was taken at 1hr interval for 5 h.

2.10.4. 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 the means were considered and not will be considered at $P \leqslant 0.01$ and $\geqslant 0.05$.

3. Results

3.1. Phytochemical screening

The phytochemical screening of the ethanolic extract of the whole plant of Enicostemma littorale revealed the presence of alkaloids, cardiac glycosides, tannins, saponins, terpenes and flavonoids.

3.2. Determination of LD_{50}

LD_{50} was calculated to be 2600.00±66.47 mg/kg. The physical signs of toxicity included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death.

3.3. Effect on suppressive activity of ethanolic crude extract and fractions of E. littorale

The extract and its fractions showed a dose-dependent chemosuppressive effect on the parasitaemia. These effects were statistically significant relative to the control ($P<0.001$). The acqeous fraction showed a high chemoinhibitory percentage of 79.95% which was comparable to that of the standard artesunate of 5 mg/kg (Table 1).

3.4. Effect on prophylactic/ repository activity of ethanolic crude extract and fractions of E. littorale

The ethanolic crude extract showed a dose-dependent reduction of parasitaemia in the extract-treated groups. These reductions were statistically significant relative to the control. The acqeous fraction demonstrated a high antiplasmodial activity which was significant ($P<0.001$) when compared to control but was uncomparable to that exhibited by the standard drug pyrimethamine of 1.2 mg/kg (Table 2).

Table 1
Suppressive activity of ethanolic leaves extract and fractions of *E. littorale* on *P. berghei* infection in mice (4-day test).

Treatments	Dose(mg/kg)	Parasitaemia	% Chemosuppression
Normal saline	10 mL/kg	21.60±1.01	–
E. littoralecrude extract	260	8.35±0.89[a]	61.34
	520	8.33±2.58[a]	61.43
	780	6.33±0.19[a]	70.69
Acqueous fraction	520	4.33±1.02[a]	79.95
Chloroform fraction	520	20.13±0.10	6.80
Artesunate	5.0	4.66±1.19[a]	78.42

Values are expressed as mean ± SEM. Significance relative to control [a]$P<0.001$, $n = 6$.

Table 2
Repository/Prophylactic activity of ethanolic leaves extract and fractions of *E. littorale* on *P. berghei* infection in mice.

Treatments	Dose(mg/kg)	Parasitaemia	% Chemosuppression
Control	10 mL/kg	15.66±0.93	–
E. littorale crude extract	260	6.33±0.68[b]	59.57
	520	5.66±0.40[b]	63.85
	780	5.33±0.45[b]	65.96
Aqueous fraction	520	4.33±1.04[b]	47.81
Chloroform fraction	520	11.00±0.26[a]	29.75
Pyrimethamine	1.2	2.66±0.93[b]	83.01

Values are expressed as mean ± SEM. Significance relative to control [a]$P<0.01$, [b]$P<0.001$, $n= 6$.

Table 3
Mean survival time of mice receiving the various doses of ethanolic leaves extract and fractions of *E. littorale* during established *P. berghei* infections in mice.

Treatments	Dose (mg/kg)	Mean survival time (day)
Normal saline	10 mL/kg	11.36±0.65
E. littorale crude extract	260	12.26±0.53[ns]
	520	20.80±0.82[a]
	780	25.63±0.78[a]
Chloroform fraction	520	16.70±0.44[a]
Acqeous fraction	520	27.33±0.66[a]
Artesunate	5.0	29.83±0.12[a]

Values are essxpressed as mean ± SEM. Significance relative to control [a]$P<0.001$, $n = 6$.

Table 4
Effect of *E. littorale* root extract on 2,4-dinitrophenol- induced pyrexia in rat.

Treatment	Dose (mg/kg)	Time interval (h)						
		Basal temperature	0.5	1	2	3	4	5
Control		36.00± 0.18	37.42±0.20	37.65±0.44	38.12±0.33	38.22±0.28	38.18±0.24	38.10±0.22
E. littorale	260	36.60± 0.55	37.48±0.47	38.28±0.23	38.45±0.51	38.30±0.33	37.90±0.48	37.73±0.32
	520	36.85±0.14	37.70±0.28	37.53±0.21	37.70±0.30	37.53±0.24	37.62±0.25	37.62±0.15
	780	36.77±0.24	37.37±0.31	37.45±0.40	37.60±0.21	37.48±0.23	37.42±0.14	36.98±0.16[a]
ASA	100	36.88±0.28	37.52±0.30	38.00±0.27	37.75±0.18	37.32±0.18	37.23±0.13	36.98±0.12[a]

Values are expressed as mean ± SEM. Significance relative to control [a]$P<0.01$, $n=6$.

Table 5
Effect of *E. littorale* root extract on amphetamine -induced pyrexia in rat.

Treatment	Dose (mg/kg)	Time interval (h)						
		Basal temp.	0.5	1	2	3	4	5
Control		36.40±0.22	37.53±0.23	37.92±0.18	38.00±0.22	38.32±0.20	38.57±0.24	38.68±0.29
E. littorale	260	36.07±0.20	37.48±0.23	37.67±0.23	37.70±0.18	37.53±0.18[a]	37.28±0.14[c]	37.17±0.12[c]
	520	36.33±0.14	37.75±0.26	37.85±0.24	37.78±0.28	37.72±0.26	37.62±0.15[a]	37.23±0.14[c]
	780	36.60±0.16	38.00±0.29	37.85±0.18	37.95±0.26	37.65±0.22	37.62±0.18[a]	37.45±0.22[b]
ASA	100	36.32±0.14	38.20±0.28	37.62±0.38	37.67±0.28	37.67±0.28	37.23±0.21[c]	37.22±0.22[c]

Values are expressed as mean ± SEM. Significance relative to control [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$, $n=6$.

3.5. Antiplasmodial effect of ethanolic crude extract and fractions of E. littorale on established infection

There was a progressive dose-dependent reduction of parasitaemia in all the extract/fraction-treated group relative to control. These reductions were statistically

Table 6
Effect of *E. littorale* root extract on yeast-induced pyrexia in rat.

Treatment	Dose (mg/kg)	Time interval (h)						
		Basal temperature	0	1	2	3	4	5
Control		36.30±0.12	37.00±0.23	37.92±0.18	38.08±0.22	38.32±0.20	38.57±0.24	38.68 ± 0.29
E. littorale	260	37.05±0.20	38.65±0.28	37.60±0.21	37.40±0.22	37.33±0.28	37.25±0.24[b]	36.85 ± 0.18[c]
	520	37.00±0.23	38.45±0.26	37.30±0.20	37.48±0.30	37.18±0.26[a]	36.95±0.15[c]	36.72 ± 0.16[c]
	780	37.00±0.25	38.43±0.15	37.97±0.12	37.92±0.24	37.80±0.25	37.47±0.22[a]	37.18 ±0.28[c]
ASA	100	36.65±0.84	38.35±0.18	37.87±0.18	37.83±0.28	37.55±0.30[b]	37.38±0.21[b]	37.13 ± 0.20[c]

Values are expressed as mean ± SEM. Significance relative to control [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$, $n=6$

significant relative to the control ($P<0.001$) (Figure 1). Though the acqeous fraction and the highest dose of the crude extract (780 mg/kg) exhibited a high chemosuppression these were uncomparable to that of the standard artesunate.

The crude extract and fractions demonstrated a significant ($P<0.001$) protective potentials on the animals as was seen in the mean survival time of the animals. The groups treated with acqeous and highest dose of the crude extract had a longer mean survival time, though less than that of the standard drug,artesunate (Table 3).

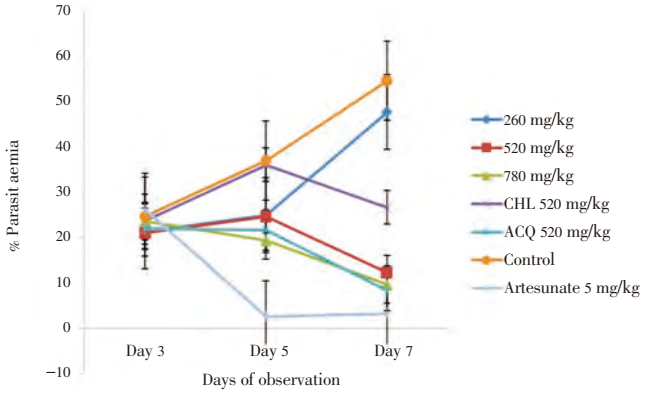

Figure 1. Curative effect of ethanolic leaves extract and fractions of *E. littorale* on *P. berghei* established infection in mice. ACQ-Acqeous fraction, CHL - Chloroform fraction.

3.6. Effect of ethanolic crude extract of E. littorale on 2,4, dinitronitrophenol (DNP)-induced pyrexia in rats

The extract (260 - 780 mg/kg) demonstrated an insignificant dose-dependent lowering of temperature in DNP-induced pyretic rats. The antipyretic effect was however pronounced ($P<0.001$) at the 5th h with the highest dose of the extract. The effect was comparable to that of the standard drug ASA (Table 4).

3.7. Effect of ethanolic crude extract of E. littorale on amphetamine-induced pyrexia in rats

Administration of the extract (260 - 780 mg/kg) of the extract produced a dose-dependent lowering of body temperatures of the treated rats against amphetamine-induced pyrexia. The effects which were significant ($P<0.05$ - 0.001) at all doses at the 4h and 5h were comparable to that of the standard ASA (Table 5).

3.8. Effect of ethanolic crude extract of E. littorale on yeast-induced pyrexia in rats

Ethanolic crude extract of *E. littorale* (260 - 780 mg/kg) exhibited a significant ($P<0.05$ - 0.001) dose-dependent lowering of rats' body temperature elevated by the administration of yeast. These effects were pronounced at the 4th h and 5th h post-treatment with extract. The antipyretic effects of the extract were comparable to that of the standard ASA (Table 6).

4. Discussion

The major folkloric uses of *E. littorale* have been in the treatment of diabetes mellitus, fever, stomach-ache, stomach ulcer, dyspepsia and malaria in western and southern India[1]. The tribal inhabitant of North Gujarat, India, use its hot water extract of the plant to treat malaria and other febrile illnesses and the *in vitro* activty of the plant has been reported. These prompted the need to evaluate the in *vivo* antiplasmodial and antipyretic potentials of the crude extract, acqueous and chloroform fractions of the whole plant of *E. littorale* to corelate with the reported *in vitro* activity and also confirm its 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. These results further confirm the reported *in vitro* activity of the plant[10]. Some secondary metabolites of plants are said to have antiplasmodial activity[12]. Swertiamarin has been reported to be a major active principle in *E. littorale*[10]. This compound has been suggested by Soni and Gupta[8] to be responsible for antimalarial activity of *Swertia chirata* which contains a lesser amount of swertiamarin than *E. littorale*. Swertiamarin, may in part, be responsible for the antiplasmodial activity observed in this study as *E. littorale* has been reported to contain a higher amount of swertiamarin than *Swertia chirata*[8]. Moreso, *E. littorale* has been reported to contain monoterpenes which have been implicated in antiplasmodial activities of plants. 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[14,15]. These could have also contributed to the antiplasmodial activity of this extract and fractions.

On antipyretic activity, the extract inhibited significantly

dinitrophenol, amphetamine and yeast-induced pyrexia. Dinitrophenol 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[16]. 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[17]. This leads to hyperthermia. Yeast induces pyrexia by increasing the synthesis of prostaglandins[18]. 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[19]. 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[20]. 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 *E. littorale* possesses considerable antiplasmodial and antipyretic activities. These confirm its use to treat malaria and fever 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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