

In-Vivo Anti-Malarial Activity of 80% Methanol Leaf Extract of *Croton Dichogamus* Pax and *Ehretia Cymosa* Thonn in *Plasmodium Berghei* Infected Mice

Desta Hashim^{1,2}, Shemsu Umer², Ariaya Hymete³, Yalemtehay Mekonnen⁴

¹Department of Pharmacology, School of Pharmacy, College of Medicine and Health Sciences, Hawassa University, Hawassa, Ethiopia; ²Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia; ³Department of Pharmaceutical Chemistry and Pharmacognosy, School of Pharmacy, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia; ⁴Department of Zoological Sciences, College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Correspondence: Shemsu Umer, Email shemsu.umer@aau.edu.et

Background: Malaria is causing high mortality and morbidity due to *Plasmodium*'s resistance to currently available anti-malarial drugs and mosquito's resistance to insecticides. Thus, there is a critical need to search for novel anti-malarial drugs from natural sources. Therefore, this study investigated in vivo antimalarial activities of two Ethiopian medicinal plants, *Croton dichogamus* Pax and *Ehretia cymosa* Thonn, in *Plasmodium berghei* infected Swiss albino mice.

Methods: Soxhlet extraction method using 80% methanol as a solvent was used to prepare crude extracts of the two plants. Acute oral toxicity and 4-day suppressive in vivo antimalarial activity tests were performed on healthy female mice and *P. berghei* infected male mice, respectively. Antimalarial activity of the crude extracts at doses of 100, 200, and 400 mg/kg and the standard drug, chloroquine were used to assess in *Plasmodium berghei* infected Swiss albino mice. Parasitemia level, packed cell volume, body weight, and rectal temperature of the mice were determined before infection (day 0) and after treatment (day 4). Survival time was determined by recording the date on which the mice died, considering the date of infection as day 0. The recorded data were analyzed using ANOVA and SPSS version 24.

Results: The result of the acute toxicity study revealed that the crude extracts were non-toxic at doses up to 2 g/kg. The extract of *E. cymosa* suppressed parasitemia level by 66.28, 63.44 and 63.14% at 400, 200, and 100mg/kg, levels while *C. dichogamus* extract suppressed parasitemia level by 45.29% at a dose of 400mg/kg. The remaining two dose levels of *C. dichogamus* extract suppressed parasitemia level by < 30%.

Conclusion: *C. dichogamus* and *E. cymosa* showed anti-plasmodial activities. *E. cymosa* exhibited a more pronounced anti-plasmodial effect than *C. dichogamus*. The activities of both plants observed in this study support their traditional use as antimalarial drugs. Further studies on these plants using solvent fractions are required to identify their active ingredients.

Keywords: malaria, antiplasmodium, croton dichogamus, ehretia cymosa, P.berghei

Background

Malaria is a febrile hemolytic disease caused by *Plasmodium* spp., including *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*¹ and is transmitted by female Anopheles mosquitoes. It is a major global public health challenge.² According to the latest World malaria report, there were 249 million cases of malaria in 2022 with 608,000 malaria deaths in 85 countries. In 2022, the WHO African Region was home to about 94% of all malaria cases and 95% of the deaths. Children under 5 years of age accounted for about 78% of all malaria deaths in the Region.³ According to the WHO, in 2022, there were 5.1 million people affected by malaria in Ethiopia, and about 75 million people were at risk of contracting the disease. In addition to its public health impact, malaria imposes a large financial burden on households.⁴

Antimalarial drug resistance mainly occurs in *P. falciparum* but now, *P. vivax* is also developing resistance to antimalarials.⁵ *P. falciparum* is resistant to almost all antimalarial drugs in South-east Asia. In Africa, *P. falciparum* clinical isolates from Equatorial Guinea were found to be resistant to artemisinin.⁶

The majority of the population in developing countries relies on plant-based medicine to treat different diseases, including malaria, particularly in Africa, owing to the cultural acceptability, inaccessibility, and unaffordability of conventional antimalarial drugs.⁷ Medicinal plants have been the source of several drugs including antimalarials such as quinine and artemisinin.^{7,8}

Several medicinal plants have been used for the treatment of malaria in Ethiopia⁹ including those that experimentally showed to possess *in-vivo* antimalarial activity against *P. berghei* infected mice, such as *Echnops kebericho*,¹⁰ *Adhatoda schimperiana*¹¹ and *Vernonia amygdalina* Del.¹²

Croton dichogamus Pax is a shrub that grows in Ethiopia, Kenya, Somalia, Rwanda, Mozambique, Tanzania, Madagascar and Uganda. It has thornless branches and green upper- and silvery-bottom-colored leaves that become bright orange when dying.^{13,14} Although, it is known as an orange-leaved croton in English,¹⁵ it has several local names in Ethiopia, including *Adaaddo* and *Ulee foonii* in Afan Oromo.¹⁶ It is widely used as a traditional medicine in East African countries for the treatment of various diseases. For instance, in Kenya, the Maasai people of the Sekenani Valley use the plant for dental hygiene¹⁷ while the Nandi people use it as a remedy for malaria (decoction of the whole plant).¹⁸ Additionally, the leaves of the plant were crushed, mixed with water, and dried orally to treat malaria, amoeba, tuberculosis, and anthrax.¹⁶ Leaf infusions are used to treat malaria in Kenya.¹⁹

Ehretia cymosa Thonn. is a small tree or shrub that grows to a length of approximately 10 m and is distributed throughout tropical Africa from Sierra Leone to Ethiopia and south to Mozambique.^{20,21} This plant is known by the common name puzzle bush or Ivorywood in Nigeria.²²

In Ethiopia, it is known by different vernacular names, such as *Game* or *Checho* in Amharic, *Garmi*, *Hulaga* and *Ulaga* in Afan Oromo, *Gidincho* in Sidama, and *Minegure* in Afar languages.^{23–25} Many parts of *Ehretia cymosa* have been used in traditional medicine in several countries to treat various diseases. For instance, the fresh leaf of the plant is crushed with water and drunk for treating “mich” or febrile illness in Ethiopia^{26,27} whereas the dried leaf aqueous decoction is drunk as a remedy for dry cough, malaria and pneumonia in Yemen.²⁸ The decoction of the leaf is used against malaria in Benin, whereas infusion of the leaf of this plant is used for viral infections such as measles and poliomyelitis in Nigeria.^{29,30} The leaf and whole plant parts of *E. cymosa* as well as phytochemical compounds isolated from the species have been reported to have antibacterial, antidiabetic, antihyperglycaemic and antioxidant activities.³¹

Although both *C. dichogamus* and *E. cymosa* have been used to treat malaria and other infections in Ethiopia and elsewhere, no scientific antimalarial studies have been conducted. Therefore, this study was designed to evaluate *in vivo* antimalarial activity of 80% methanolic extract from the two plants in *P. berghei* infected mice.

Methods

Collection, Preparation and Extraction of Plant Material

Fresh mature leaves of both *C. dichogamus* Pax and *E. cymosa* Thonn (at the seeding stage) were collected from their natural habitat around the Abjata-Shalla Lakes National Park in Nagele Arsi District of West Arsi Zone, Oromia Region, which is located 230 km southeast of Addis Ababa and 25 km north of Shashemane.

The plants were identified and authenticated by a botanist Mr. Melaku Wondafrash and sample specimens were deposited in the National Herbarium of Addis Ababa University, Ethiopia with voucher numbers DH001 (*E. cymosa*) and DH002 (*C. dichogamus*). Cleaned, dried, and powdered plant samples were Soxhlet extracted using 80% methanol. Extracts dried to a semisolid powder were stored at -20°C in amber-colored bottles until use.

Acute Toxicity Test

An acute oral toxicity test was performed to determine the median lethal dose (LD_{50}) of the extracts, which helped fix the dose level to be administered to laboratory animals. In this study, nulliparous and non-pregnant female Swiss albino mice weighing 25–30 g and 8–10 weeks of age were arbitrarily recruited according to the Organization for Economic Corporation and Development (OECD) guideline 425³² to evaluate the acute toxicity of plant extracts. Five (5) mice were used for each plant. The mice were kept under standard laboratory conditions and marked on their tails for easy identification. First, a single dose of 2000 mg/kg each of *C. dichogamus* and *E. cymosa* extract was administered orally to

two mice (one mouse per extract) that were fasted for 4 h before and 2 h after administration of the extract, but had access to a sufficient amount of tap water. After extract administration, mice were monitored continuously for the first 4 h and intermittently for 24 h. Any observed signs of acute toxicity such as changes in appetite, skin fur, eye secretions, salivation, diarrhea, lethargy, sleep, convulsions, coma, and death were recorded. When no deaths or signs of over-toxicity were observed in the initial two mice within 24 h, an additional four female mice for each plant extract were administered similarly and followed for 14 days on a daily basis to assess the delayed toxicity of the plant extracts.^{33,34}

Handling, Grouping and Dosing of the Mice

Healthy Swiss albino male mice of 8–10 weeks of age and 22–35 g of weight were purchased from Ethiopian Public Health Institute (EPHI), kept in Animal House of College of Health Sciences, Addis Ababa University, Ethiopia and used for the malaria 4-day suppressive test. The animals in standard cages were acclimatized for seven days to the laboratory environment and experimental conditions by carefully inserting a feeding gavage into and then removing their mouth prior to the commencement of the experiment to minimize the stress that may occur during the experiment. Animals were provided a standard pellet diet and water *ad libitum* with a 12 h light/day cycle and optimum temperature and humidity.^{33,35}

A total of 50 mice (25 per plant) were randomly divided into five groups of five mice, including three treatments and two control (positive and negative) groups. Three dose levels (100, 200, and 400 mg/kg) of each plant leaf extract dissolved in 5% DMSO were administered to the *P. berghei* infected mice. The Mice in the negative control group received 5% DMSO at 10mL/kg while those used for positive control received Chloroquine at 25mg/kg.^{36,37}

Maintenance and Inoculation of Plasmodium Berghei

A donor mouse infected with *P. berghei* ANKA-65 strain was obtained from Mekele University, Ethiopia in September 2019. After the mouse arrived at Addis Ababa University, Ethiopia, the parasitemia level was determined by drawing 1 mL blood from the heart via cardiac puncture. To maintain the parasite, the blood was diluted and injected into healthy donor mice. Inoculum was prepared using infected blood (1mL) obtained from a mouse with 36% parasitemia via cardiac puncture, diluted to 1×10^7 iRBCs/ 0.2mL of blood. Experimental mice were intraperitoneally inoculated with 0.2 mL of infected blood and kept in one large cage for approximately 2 h until use in the study.^{34,36}

The Peter's Four-Day Suppressive Test

The chemo-suppressive activity of the plants was evaluated in mice infected with *P. berghei* parasites using a standard four-day suppressive test model. Body weight, rectal temperature, and packed cell volume (PCV) were measured on day 0 prior to infection in all mice. Three hours post-infection, the three experimental groups of mice were treated with *C. dichogamus* and *E. cymosa* extracts at doses of 100, 200, and 400 mg/kg. The negative and positive control groups' mice were given 5% DMSO and 25mg/kg chloroquine, respectively. Treatment was continued for 4 consecutive days, from day 0 to day 3.^{37,38}

Determination of Parasitemia Level

On day 4 (96 h post infection), blood was collected from the tail of each mouse, smeared on microscope slides, fixed with absolute methanol for 5 min, stained with 10% Giemsa stain for 15 min, washed carefully with tap water, and left to dry at room temperature. The number of *P.berghei* infected red blood cells (iRBC) on the slides was counted using a microscope at 100X magnification. The level of parasitemia was determined by averaging the counts of the three fields on each slide. The percentage of chemosuppression was determined as the percentage of parasitemia in the negative control and treatment groups.³⁶

$$\% \text{ parasitaemia} = \frac{\text{Number of parasitized RBC}}{\text{Total number of RBC counted}} \times 100$$

$$\% \text{ parasitemia suppression} = \frac{\text{Parasitemia (negative control - treatment group)}}{\text{Parasitemia in negative control}} \times 100$$

Determination of Packed Cell Volume (PCV)

PCV was measured to assess the ability of the crude extracts to prevent the destruction of red blood cells (hemolysis) arising from increasing levels of *Plasmodium* parasite in the bloodstream. Blood was drawn from the tail of each mouse using heparinized capillary tubes, where heparinized ends were filled with blood to 3/4th of their height. The capillary tubes, with carefully sealed non-heparinized ends using sealing clay, were placed in a centrifuge with the sealed ends facing outwards, and centrifuged at 12,000 rpm for 5 min¹².

$$\text{Packed cell volume (PCV)} = \frac{\text{volume of erythrocytes in a given volume of blood}}{\text{Total blood volume}}$$

Determination of Body Weight and Rectal Temperature

The body weight and rectal temperature of each mouse were measured daily from the time of infection (day 0) to the last day of treatment (day 4).³⁹ Body weight and rectal temperature were measured to determine the ability of the extracts to prevent the body weight loss and temperature decrease anticipated in rodent malaria infections.^{12,40}

Determination of Mean Survival Time (MST)

MST was measured by recording the mortality of each mouse daily to assess the antiplasmodial activity of the extracts. The number of days from the time of inoculation (infection) to death (if any) of each mouse was recorded during the follow-up period (60 d). Mice were allowed free access to food and water. An extract that resulted in a longer survival time compared to the control group was considered active.⁴⁰ The following formula was used to determine the MST of experimental mice.³⁴

$$\text{MST} = \frac{\text{Sum of survival time of all mice in a group (days)}}{\text{Total number of mice in that group}}$$

Method of Data Analysis

The data obtained were analyzed using the Statistical Package for Social Sciences (SPSS) version 24 and are presented as mean \pm SEM. The difference between groups in the experiment was determined by one-way analysis of variance (ANOVA) followed by a post-hoc Tukey HSD Multiple comparison test. Differences were taken as significant at 95% confidence interval (P value < 0.05).

Ethical Consideration

The experimental animals were handled ethically following the OECD 145 guidelines and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. For this research, ethical approval and clearance with the protocol number ERB/SOP/201a/12/2019 was obtained from Institutional Review Board (IRB) of School of Pharmacy, College of Health Sciences, Addis Ababa University, Ethiopia.

Results

Percentage Yields of Crude Extract

Black gummy semi-solid (22%) and a dark brown colored solid substance (16%) were obtained from 80% methanol crude leaf extracts of *C. dichogamus* and *E. cymosa*, respectively.

Acute Toxicity

In the acute toxicity assays conducted by administering a limit dose of 2000 mg/kg of the plant leaf extracts, no mortality or signs of over toxicity were recorded within the two-week period of observation.

The 4 - Day Suppressive Test for Antimalarial Activity

An early malaria infection assay (4-day suppressive test) revealed that *C. dichogamus* has moderate anti-plasmodial activity against *P. berghei*. *C. dichogamus* extract showed parasitic chemosuppression of 24.64, 27.77 and 45.29% at 100, 200, and 400 mg/kg/day dose levels, respectively, compared with that of the negative control (Table 1). At all evaluated dose levels, the extract exhibited a statistically significant decrease in parasitemia ($p < 0.001$) compared to the negative control. The observed suppression was lower than that of the standard drug chloroquine.

The findings of this study showed that, the 80% methanol extract of *E. cymosa* had strong antiplasmodial activity against *P. berghei*. *E. cymosa* extracts at 100, 200, and 400 mg/kg/day significantly suppressed parasitemia level by 63.14, 63.44, and 66.28%, respectively (p value < 0.001).

All dose levels of *C. dichogamus* extract did not significantly prolong survival time compared with vehicle-treated animals. The chloroquine-treated group exhibited a statistically significant ($p < 0.001$) prolongation in survival time. In the case of *E. cymosa*, the higher dose of the extract (400 mg/kg/day) exhibited significant prolongation ($p < 0.05$) of survival time compared to the negative control (Table 1).

Evaluation of the body weight of the mice before and after treatment with *C. dichogamus* revealed that the extract did not significantly prevent a decrease in body weight at any dose level. *E. cymosa* leaf extract prevented body weight reduction compared to the negative control. The prevention of body weight loss by the three doses of the extract was comparable to that of the standard drug.

Analysis of percent PCV change between days 0 and 4 indicated that the higher dose level of *C. dichogamus* (400 mg/kg) significantly ($p < 0.01$) prevented reduction in PCV compared to the negative control. The lower dose (100 mg/kg) did not avert PCV reduction to a statistically significant level compared with that of the negative control. Similarly, *E. cymosa* extract at all dose levels (400, 200, and 100 mg/kg) prevented PCV reduction to a significant level compared with that of the negative control (Table 2).

All dose levels of *C. dichogamus* extract significantly prevented a decrease in rectal temperature on day 4 ($p < 0.05$) compared with the negative control. However, its preventive effect was not comparable to that of the positive controls.

Discussion

The findings of this study revealed that the plant extracts under investigation were safe for use at doses lower than 2000 mg/kg, as no death or signs of toxicity were observed during the acute toxicity studies in the mouse model. The results of the current study on *C. dichogamus* crude extract at 400 mg/kg dose level indicated that, it reduced parasitemia level by 45.29%. This finding provides evidence of the anti-plasmodial activity of the leaves of the plant during malaria infection. On the contrary, the lower doses (200 and 100 mg/kg) produced weak (27.77% and 24.64%, respectively) parasitemia suppressions. According to one study, at least 30% parasitemia suppressive activity is required for an extract to be considered as having anti-plasmodial activity.⁴¹

Table 1 Parasitemia, Percent Chemosuppression and Survival Time of *P. berghei* Infected Mice Treated with 80% Methanol Leaf Extract of *C. dichogamus* and *E. cymosa* in the 4-Day Suppressive Test

Test substance (mg/kg)	<i>C. dichogamus</i>			<i>E. cymosa</i>		
	Parasitemia level	% suppression	Survival time (days)	Parasitemia level	% suppression	Survival time (days)
NC (5%DMSO)	79.18±2.41	–	13.00±0.89	73.16±1.27	–	11.00±1.64
100mg/kg/day	59.68±4.11 ^{a2b3c2}	24.64	14.00± 0.95 ^{b3}	26.97±3.19 ^{a3b3}	63.14	16.40±1.12 ^{b3}
200mg/kg/day	57.19±1.66 ^{a3b3c1}	27.77	14.20± 0.92 ^{b3}	26.75±3.27 ^{a3b3}	63.44	18.00± 0.95 ^{b3}
400mg/kg/day	43.32±4.31 ^{a3b3d2e1}	45.29	17.20±1.85 ^{b3}	24.67±3.86 ^{a3b3}	66.28	21.80±1.46 ^{a1b3}
PC (CQ 25mg/kg/day)	0.00±0.00	100	38.60±3.75	0.00±0.00	100	41.20±3.71

Notes: Results are presented as mean ± SEM; n = 5; a = compared to negative control, b = compared to CQ25 mg/kg, c = 400 mg/kg, d = 200 mg/kg, e = 100 mg/kg; 1 $p < 0.05$, 2 $p < 0.01$, and 3 $p < 0.001$.

Abbreviations: CD, *Croton dichogamus* crude extract; EC, *Ehretia cymosa* crude extract; CQ, chloroquine; DMSO, dimethyl sulfoxide; NC, negative Control; PC, positive control.

Table 2 Body Weight, Rectal Temperature and Packed Cell Volume in *P. berghei* of Infected Mice Treated with 80% Methanol Leaf Extracts of *C. dichogamus* and *E. cymosa* in the 4-Day Suppressive Test

Test substance (mg/kg)	<i>C. dichogamus</i>									<i>E. cymosa</i>								
	Body Weight			Rectal temperature			Packed cell volume			Body Weight			Rectal temperature			Packed cell volume		
	Day 0	Day 4	% change	Day 0	Day 4	% change	Day 0	Day 4	% change	Day 0	Day 4	% change	Day 0	Day 4	% change	Day 0	Day 4	% change
5%DMSO	30.78 ±1.84	29.41 ±1.96	-4.45	37.36 ±0.37	36.34 ±.34	-2.73	61.92 ±1.29	57.14 ±2.99	-7.72	26.07± 0.85	25.14 ±0.59	-3.57	36.28 ±0.22	34.84 ±0.31	-3.97	61.35 ±2.08	56.85 ±4.17	-7.33
100	30.44 ±1.11	30.29 ±1.80	-0.49	37.02 ±0.25	36.56 ±.37	-1.24	66.79 ±3.41	67.49 ±2.58	1.04	27.51± 0.98	29.83 ±1.29	8.43	37.28 ±0.12	37.08 ±0.25	-0.40 ^{a3}	60.57 ±1.20	64.11 ±1.95	5.84
200	30.12 ±1.68	30.19 ±1.39	0.23	36.72 ±0.18	36.32 ±.25	-1.09	62.42 ±3.17	63.13 ±2.47	1.14	27.34± 1.37	30.02 ±1.99	9.80	36.58 ±0.25	36.96 ±0.17	1.04 ^{a3}	63.78 ±1.34	68.99 ±2.31	8.17
400	29.89 ±1.30	31.04 ±1.43	3.85	36.94 ±0.49	36.76 ±.37	-0.49	68.72 ±2.18	70.04 ±1.88	1.92 ^{a2}	25.51± 1.10	28.02 ±1.09	9.84	36.44 ±0.18	37.02 ±0.21	1.59 ^{a3}	55.07 ±3.06	60.03 ±1.78	9.01
CQ 25	28.89 ±1.26	31.94 ±1.49	10.56	36.60 ±0.59	36.70 ±.32	0.27	67.69±2. 90	71.31 ±3.49	5.35	26.02± 1.06	28.64 ±1.21	10.07	35.66 ±0.25	36.54 ±0.21	2.47	59.00 ±4.30	69.66 ±3.54	18.07

Notes: Results are presented as mean ± SEM; n = 5; a = compared to negative control, b = compared to CQ 25 mg/kg, c = 400 mg/kg, d = 200 mg/kg, e = 100 mg/kg; 2 p < 0.01.

Abbreviations: CD, *Croton dichogamus* crude extract; EC, *Ehretia cymosa*; CQ, chloroquine; DMSO, dimethyl sulfoxide; D0, pre-treatment value on day 0; D4, post-treatment value on day 4.

Similarly, *E. cymosa* extracts at doses of 400, 200, and 100 mg/kg demonstrated chemosuppression of 66.28, 63.44, and 63.14%, respectively. All doses of the extract resulted in statistically significant parasitemia suppression ($P < 0.001$) compared to both the negative and positive control mice. For *in-vivo* antimalarial studies, activity of an extract is classified as very good, good, or moderate if it suppresses parasitemia by $\geq 50\%$ at respective dose levels of 100, 250, and 500 mg/kg (Tajbakhsh et al, 2021). Accordingly, the chemosuppressive activity observed for *E. cymosa* extract can be categorized as very good.

Survival time prolongations observed for all dose levels of *C. dichogamus* extract were not significant compared with those of the negative control. *E. cymosa* extract, compared to the negative (11.00 ± 1.64) and positive control, showed significant enhancement ($p < 0.001$) of survival time as mice in all dose groups survived averagely for 21.80 ± 1.46 , 18.00 ± 0.95 and 16.40 ± 1.12 days. The observed prolongation of survival was presumed to be the result of the excellent chemosuppressive capacity of the extract.

A decrease in body weight, reduction in body temperature, and anemia due to lysis of red blood cells are characteristic features of malaria infection in mice. Therefore, plant-derived antimalarial agents are expected to reduce parasitemia levels and thus prevent decreases in both body weight loss and PCV, while maintaining normal body temperature at normal level.⁴² In this study, *C. dichogamus* extract at doses of 400 and 200 mg/kg caused a slight gain in body weight compared with the negative control. This increase in body weight could be associated with the likelihood that the extract might enhance the appetite of treated mice or the weight loss induced by the infection is reverted because of the decrease in parasitemia.

Malaria infection causes anemia in both humans and mice owing to the destruction of infected RBCs, clearance of infected and uninfected RBCs, and suppression of erythropoiesis.³⁹ *C. dichogamus* extract at a dose of 400 mg/kg significantly prevented the decrease in PCV ($p < 0.01$). None of the doses of *E. cymosa* extract significantly prevented a decrease in PCV compared to that in untreated mice.

Fever is a classic symptom of malaria in humans, in contrast to hypothermia, which is a typical feature of malaria in mouse models. This difference is due to the large surface area-to-body mass ratio of small animals, such as mice, which facilitates a higher degree of heat loss and prevents the development of fever caused by pyrogenic agents.⁴³ In the present study, the rectal temperature was measured to evaluate the ability of the extracts to counteract hypothermia. In this study, all dose levels of *C. dichogamus* slightly prevented rectal temperature decreases compared with the negative control, but the average temperature reduction was not as strong as that of the standard drug. This finding is consistent with that of a similar study on *Cordia africana* in Ethiopia.³⁷ In contrast to *C. dichogamus*, the higher and middle doses of *E. cymosa* extract significantly prevented a decrease in rectal temperature compared with the negative control ($p < 0.001$). A lower dose of the extract did not prevent a decrease in the rectal temperature. The results obtained for both plant extracts during antimalarial activity testing in the current study strengthen the traditional claim of their use to treat malaria and other febrile conditions.

Several phytochemicals have been identified from the leaves, roots and whole plant parts of *E. cymosa* including alkaloids, anthraquinones, essential oils, fatty acids, flavonoids, glycosides, phenolics, proanthocyanidins, pseudotannins, reducing sugars, saponins, steroids, tannins and terpenes.³¹

Among the reported secondary metabolites in both plants, alkaloids are well-known for their activity against various protozoans, including malaria parasites. Compounds with antioxidant activity, such as alkaloids, inhibit heme oxidation, which the parasite uses for heme polymerization (conversion of host hemoglobin into hemozoin), because unpolymerized heme is lethal for the survival of plasmodia in red blood cells.^{44,45}

The antimalarial activities of triterpenoids and sesquiterpenes could be related to their capacity to produce free radicals inside the parasite, which alkylate macromolecules, disrupt the cell membrane, and interfere with the intracellular transport of the parasite. Malaria infection causes increased oxidative stress manifested by the generation of free radicals in the host cell, and antimalarial activity may be due to tannins (antioxidants) in the plant extract that scavenge free radicals.⁴⁵

Conclusion

It can be concluded from the results of this study that *E. cymosa* and *C. dichogamus* leaves possess *in-vivo* antiplasmodial activity in mice. Further studies, such as fractionation, isolation, and structural elucidation of the active phytoconstituents of both plants, are needed.

Acknowledgments

The Authors would like to thank Addis Ababa University and thematic Project PI for their financial support. The authors would also like to acknowledge that the MSc thesis from which this manuscript was extracted is available online at the Addis Ababa University Institutional Repository (AAU-ETD).

Disclosure

The authors report no conflicts of interest in this work.

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