Evaluation of *in vitro* and *in vivo* Antiplasmodial Activity of the Leaf Latex of Aloe Weloensis (Aloaceae)

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Research

Keywords: Malaria, Aloe weloensis, Plasmodium berghei, Mice

Posted Date: June 26th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-37849/v1

Abstract

Background: Nature has gifted a variety of plants having potential effect against plasmodium parasites. The present study was aimed to determine *in vitro* and *in vivo* antimalarial activity of the leaf latex of *Aloe weloensis*.

Methods: *In vitro* antimalarial activity of the leaf latex of *A. weloensis* was determined against 3D7 strain of *P. falciparum*. Antimalarial activity of the three doses the latex was evaluated in 4 day-suppressive and curative models against *P. berghei* infected mice. Antioxidant activity of the leaf latex of *A. weloensis* was assessed in 2,2- diphenyl 1- picrylhydrazine assay model.

Results: Antioxidant activity of the latex was concentration dependent; the strongest inhibition was measured at 400 µg/mL (73.54%). The leaf latex of *A. weloensis* was demonstrated inhibitory activity against 3D7 malarial strain (IC50 = 9.14 µg/ml). Suppressive and curative effect of the latex was found to be dose dependent. Parasitemia reduction was significant (200 mg/kg, $p<0.01$, 400 and ,600 mg/kg, $p<0.001$) in 4-day suppressive test compared to vehicle control. Parasitemia level of the mice treated with 200, 400 and 600 mg/kg doses of the latex significantly ($p<0.001$) reduced with suppression of 36%, 58% and 64% respectively in curative test. Administration of the leaf latex of *A. weloensis* significantly ($p<0.01$) improved mean survival time, pack cell volume, rectal temperature and body weight of *P. berghei* infected mice.

Conclusion: The finding showed that the leaf latex of *Aloe weloensis* endowed prominent antimalarial and antioxidant activities. The result can serve as a step towards the development of safe and effective herbal therapy against plasmodium parasites.

Introduction

Herbal medicines are proven safe and effective for healing of diseases and have been the potential source for the development of noble drugs (1, 2). The majority of people in the world relies on plant medicine healing modalities (1, 3). Phytotherapy is the antecedent of modern drugs as isolated active compounds are vital for development of modern drugs and one-third of the top-selling drugs are plant origins (4–6). Currently available antimalarial drugs like quinine, halofantrine, mefloquine, chloroquine and more recently arthimisinin are plant origin (7–9).

Lack of available vaccines and emerging of resistance on antimalarial drug have provided the necessity to find noble plant based antimalarial drugs (6, 10, 11). Developing noble antimalarial agents are imperative in order to overcome challenges posed by the development of antimalarial drug resistance. Nature has gifted a variety of plants having potential effect against plasmodium parasites (12–14). Medicinal plants are the major resource for the treatment of malaria infections in Africa since health care facilities are limited (15).

Aloe species have been used for as topical and oral therapeutic agents due to their health, beauty, medicinal, and skin care properties. They have been demonstrated antibacterial, antitumor, anti-inflammatory, anti-arthritic, anti-rheumatoid, anticancer and antidiabetic activities (16). The latex of *Aloe weloensis* leaf showed antibacterial effect against gram negative and gram positive strains (17). The leaf latex of the plant has been used in folk medicine against malarial and others human ailment in Ethiopia (18).The leaf latex and isolated compounds of *Aloe vera*, *Aloe pulcherrima* and *Aloe megalacantha*, a similar plant species, possessed significant antimalarial activity (19–21).As a result, *Aloe weloensis* may have similar effect as all plants belong to the same genus. Therefore, the current study was aimed to investigate antimalarial activity of the leaf latex of *Aloe weloensis.*

Methods

Drugs, chemicals and Materials

Chloroquine (Julphar), DPPH (97% Japan Tokyo), normal isotonic saline, sodium citrate, pentobarbitone, dimethyl sulfoxide, 5% sorbitol, 10% Giemsa stain, distilled water, tween-80, 1 ml syringes with needles, feeding tube, vials, gloves, 70% ethanol, ferric chloride, sodium hydroxide, hydrochloric acid, concentrated H_2SO_4 and Wagner's reagent (Iodine in Potassium Iodide) were used for the experiment.

Plant collection and preparation of the leaf latex of *Aloe weloensis*

The leaf of *Aloe weloensis* was collected from Gubalafto (North East, Ethiopia) in February, 2019. Identification of the plant was carried out by a professor Sebsebe Demisew and a specimen of the plant material was deposited in the National Herbarium of Addis Ababa University (AAU) with voucher specimen number of SD010/11. The leaf of *Aloe weloensis* was cut transversely close to the stem and then, leaf was inclined towards collecting plate to obtain yellowish exudate. The latex was dried under shade at room temperature with optimal ventilation. The dried latex was kept in clean vial and stored a desiccator until used for the experiment

Experimental animals and parasite

Healthy Swiss albino mice either sex (20-35 gram) and 2–3monthes) were used in the study. The mice were gained from Wollo University and kept in the animal house of pharmacy department in 12 h light -12 h dark cycle and permitted free to diet and water *ad libitum* (22). Animals were acclimatized to the laboratory conditions for one week before the initiation of the experiment. *Plasmodium berghei* ANKA strain was obtained from EPHI. The parasite was maintained by serial passage of blood from infected mice to uninfected ones on seven days basis. This study was carried out based on the guide for the care and use of laboratory animals (23-25).

Phytochemical screening

Standard screening tests on the leaf latex was carried out to determine various plant constituents. The latex was screened for the presence of alkaloids, anthraquinones, flavonoids, glycosides, phenols, saponins and tannins compounds by using standard procedures (26, 27).

Acute oral toxicity study

Acute oral toxicity study was carried out based on OECD guidelines 425 (28). One female Swiss albino mouse was fasted for 4 hours and the fasting body weight of the animal was measured. Then, the leaf latex was administered to mouse at a dose of 2000 mg/kg. Then, mouse was kept under strict observation of physical and behavioral changes for one day, with special attention during the first four hours. Following the result from the first mouse, another four mice were fasted for 4 hours and then, the latex was administered to each mouse at the dose of 2000 mg/kg and were observed in the same manner. The observation was continued for fourteen days for any signs of overt toxicity and based on toxicity result; the dose of the latex was determined for experimental study.

In vitro antioxidant activity of the leaf latex *Aloe weloensis*

Antioxidant activity of the latex of *Aloe weloensis* leaf was evaluated by using DPPH free radical scavenging assay (29). A weight of 4 mg of DPPH was dissolved in 100 ml methanol in the dark and 3.9 mL of a 0.1 mM methanolic solution of DPPH was mixed with a 0.1 ml methanolic solution of different concentrations (12.5–400 µg/ml) of the latex and incubated in the dark for thirty minutes at room temperature. Ascorbic acid used as standard antioxidant. After thirty minutes, the absorbance of the mixture and the control at 517nm were read by using a UV spectrophotometer. The test was carryout in triplicate and the percent of scavenging of inhibition of was calculated as:

$$\% \text{ free radical scavenging} = \frac{A - B}{A} \times 100$$

A; the absorbance of the control, B absorbance of the latex or ascorbic acid

In vitro antimalarial evaluation of the leaf latex of *Aloe weloensis*

Chloroquine sensitive *P. falciparum* (3D7 strain) was used *in vitro* blood stage culture to determine antimalarial efficacy of *Aloe weloensis*. *Plasmodium falciparum* culture was maintained in the method describe with some modification (30, 31). *Plasmodium falciparum* (suspension of 3D7) synchronized in 5% sorbitol to ring stage was seeded (200 µl/well with 2% ring stages and O^{Rh+} red blood cells at 2% hematocrit) in 96-well tissue culture plates. Then, latex of *A. weloensis* leaf in different concentrations (10 - 320 µg/ml) was added to these wells. Chloroquine at the same concentration was used as the standard control and dimethyl sulfoxide without the tested samples were used as the negative control. The parasites were cultured for 30h in the desiccators and then, incubated at 37°C for 72h in 2% O_2, 5% CO_2

and 93% N_2 (22, 31). The infected RBCs were transferred into freshly prepared complete medium to propagate the culture. After 72h incubation, the cultures were preserved at - 20 °C and the parasites were harvested. The thin blood smears were prepared and fixed with 100%methanol and stained with 10% Giemsa for 30munite to evaluate the growth stage of the parasites. The parasitemia was examined under the microscope and IC50 was determined by plotting concentration of the latex on percentage of growth inhibition. Percentage growth inhibition of the parasites was determined by using the following formula (22, 30).

$$\% \text{ of growth inhibition} = \frac{\text{Mean parasitemia of the control} - \text{Mean parasitemia of the sample} \times 100}{\text{Mean parasitemia of control}}$$

Parasite inoculation

Plasmodium berghei ANKA strain was used for induction of malaria in experimental mice. The parasites were maintained by intraperitoneal serial passage of blood and parasitemia level (30-37%) of *P. berghei* infected donor mice were determined (32, 33). Donor mouse was anaesthetized by pentobarbitone at 150 mg/kg i.p. and infected blood was collected by cardiac puncture into heparinized vacutainer tube containing trisodium citrate (0.5%). The blood was then diluted in normal saline (0.9%) and RBC count of normal mice so that the final suspension would contain about 1×10^7 parasitized red blood cells (PRBCs) in 0.2ml suspension (22, 23). Each mouse used in the study was infected intraperitoneally with 0.2ml containing 1×10^7 *P. berghei* parasitized RBCs.

Dosing and grouping of the animals

The mice were divided to five groups randomly (n=6). Group I (negative control) was treated with 10 mg/kg 2% Tween-80 in distilled water (TW80); Group II, III and IV were treated with 200 ,400 and 600 mg/kg doses of the leaf latex respectively and Group V was treated with the standard drug, chloroquine (25 mg/kg) (23, 24).

Antimalarial activity of the leaf latex of *A. weloensis* in 4-days suppressive test (Peter's test)

Randomly assigned mice were inoculated with parasite and after three hours inoculation, infected mice were treated according to their respective grouping once daily for four days. On day five, blood samples were collected from tip tail of each mouse and slides were prepared. Then % inhibition, parasitemia level and survival time was determined.

Antimalarial activity of the leaf latex of *A. weloensis* in curative test (Rane's test)

On the first day (day 0), the mice were injected intraperitonially with standard inoculum of 1x 10^7 *P. berghei* infected erythrocytes. After seventy-two hours, mice were randomly assigned into five group (n=6). Group I was treated with vehicle; group II, III and IV were treated three doses of the latex of *A. weloensis* respectively; group V was treated with chloroquine daily for 5 days. Thin blood films were prepared from tail blood of each mouse daily for five days to determine the levels of parasitemia and Mean survival time for each group (22, 23, 33).

Peripheral blood smears preparation

Thin smears of blood were made from the tail of each mouse on the fifth day (D_4). The smears were applied on microscopic slides and the blood was drawn evenly across a second slide to make thin blood films and allowed to dry at room temperature. Then they were fixed with 100 % methanol and stained with 10 % Giemsa stain (pH = 7.2) for 15 minutes.

Parasitemia determination

Each stained slide for each mouse was examined under microscope. The parasitemia level was determined by counting the number of parasitized erythrocytes in random fields of the microscope. Percent parasitemia and percent suppression were calculated by using the following formula, respectively.

$$\% \text{ Parasitemia} = \frac{(\text{number of parasitized RBC})}{(\text{total number of RBC})} \times 100$$

$$\% \text{ Suppression} = \frac{(\text{mean parasitemia of negative control-mean parasitemia of treated group})}{(\text{mean parasitemia of negative control})} \times 100$$

Determination of mean survival time

Mean survival time (MST) is another parameter that is commonly used to evaluate the efficacy of antimalarial plant materials. Mortality was monitored every day and the number of the days from the time of infection up to death was recorded for each mouse in the treatment and control groups throughout the follow-up period and the MST was calculated for each group by using the following formula.

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

Packed Cell Volume Measurement

The packed cell volume (PCV) was measured to predict the effectiveness of the test latex in preventing hemolysis resulting from increasing parasitemia associated with malaria. Blood was collected from the tail of each mouse in heparinized micro hematocrit capillary tubes by filling three-quarters of its volume. The tubes were sealed by sealant and placed in a micro hematocrit centrifuge with the sealed ends outwards.

The blood was then have centrifuged at 12,000 rpm for 15 min. The tubes were then taken out of the centrifuge and PCV were determined using a standard Micro-Hematocrit Reader, the PCV of each mouse was then measure before infection and on day four after infection using the formula (23, 24, 32).

PCV = (Volume of erythrocytes in a given volume of blood)

(Total blood volume)

Determination of body weight and temperature changes

The body weights of the mice were determined to observe whether the leaf latex was prevented weight loss for Peter's test, the body weight of each mouse was measured before infection (day 0) and on day 4 using a sensitive digital weighing balance. Rectal temperature was also measured by a digital thermometer before infection, and four hours after infection and then daily.

Statistical analysis

The results of the study were expressed as the mean ± standard error of the mean. Statistical analysis of the data was carried out with one-way analysis of variance followed by Tukey post hoc multiple comparison test. Significant differences were set at p values lower than 0.05.

Results

Phytochemical study

Preliminary phytochemical screening showed that the leaf latex of *Aloe weloensis* contained secondary metabolites (Table 1).

Table 1
Preliminary phytochemical screening of the leaf latex of *Aloe weloensis*

Phytomolecules	Result
Phenol	+
Anthraquinones	+
Glycosides	+
Alkaloids and	+
Flavonoids	+
Tannins	+
Terpenoids	-
Saponins	+
Key: + Presence, - Absence	

Acute Toxicity

In acute toxicity studies, no sign of toxicity or mortality was observed in mice after oral administration of the leaf latex at single dose of 2000 mg/kg with two weeks, signifying that the LD_{50} was greater than 2000 mg/kg.

Antioxidant activity of the leaf latex of Aloe weloensis

Antioxidant capacity of the latex was assayed using DPPH free radical. Qualitative detection showed that the color of test solution changed from violet to slightly yellow color. The finding of the study showed antioxidant activity of the latex of *A. weloensis* leaf was concentration dependent with IC50 value of 14.25 µg/ml (Table 2).

Table 2
Percentage of free radical scavenging activity of the leaf latex of *Aloe weloensis*

Concentration (µg/ml)	% of DPPH Inhibition		IC50 (µg/ml)	
	AA	LL	AA	LL
12.5	16.03 ± 0.16	7.21 ± 0.37		
25	27.82 ± 0.23	11.67 ± 0.71		
50	44.56 ± 0.52	23.14 ± 0.53	3.97	14.25
100	70.21 ± 0.32	37.01 ± 0.41		
200	79.38 ± 0.45	58.01 ± 0.31		
400	90.13 ± 0.34	73.54 ± 0.27		

Note: The results express as mean ± standard error of the mean. n = 3. AA, Ascorbic acid; IC, Inhibitory concentration; LL, Leaf latex.

The effect of the leaf latex of A. weloensis **on** P. falciparum **growth in culture**

After 72hr incubation, the latex of *Aloe weloensis* was potently inhibit the growth of *Plasmodium falciparum* (3D7 strain). The finding showed that the latex was active against *P. falciparum* parasites and growth inhibition was concentration dependent (Fig. 1). The IC50 of the latex and chloroquine were found to be 9.14 and 0.12 µg/ml respectively.

The effect of the leaf latex of Aloe weloensis **in the 4-day suppressive test**

The finding revealed that the leaf latex at the doses of 200 mg/kg ($p < 0.01$),400 and 600 mg/kg ($p < 0.001$) significantly reduced parasitemia level in four-day suppressive test compared to the vehicle control. Parasitemia reduction was dose dependent and % suppression was increased with increased the doses of the latex of *Aloe weloensis* leaf (Fig. 2). At the same time, all doses of *A. weloensis* leaf latex significantly ($p < 0.01$) increased mean survival time of the mice. Survival time of 200 mg/kg dose of the latex significantly ($p < 0.05$) lower than 25 mg/kg chloroquine ($p < 0.001$).

The effect of the leaf latex of A. weloensis **on PCV, rectal temperature and body weight**

In this study, the leaf latex at 400 and 600 mg/kg doses significantly ($p < 0.01$) prevented packed cell volume and reduction rectal temperature of *P. berghei* infected mice with respect to the vehicle control. In addition, 25 mg/kg chloroquine was significantly ($p < 0.001$) prevented PCV and rectal temperature (Table 3). Prevention of body weight reduction was significant ($p < 0.05$ at 200 and $p < 0.01$ at 400 and 600 mg/kg in the latex treated mice compared to the vehicle in the 4-day suppressive test.

Table 3
Packed cell volume, rectal temperature and body weight of infected mice treated by the leaf latex of *A. weloensis* in the 4-day suppressive test

Groups	PCV		Temperature		Body weight	
	Day 0	Day 4	Day 0	Day 4	Day 0	Day 4
10 ml/kg NC	49.60 ± 1.36	40.60 ± 1.81	37.12 ± 0.16	29.64 ± 0.36	28.80 ± 0.37	24.760 ± 0.23
25 mg/kg CQ	48.12 ± 0.10	53.20 ± 0.37^{a3b1}	35.60 ± 0.73	37.42 ± 0.12^{a3}	27.60 ± 0.51	32.60 ± 0.73^{a3}
200 mg/kg LL	48.00 ± 0.71	42.00 ± 0.95^{c1}	36.58 ± 0.13	32.38 ± 0.31^{c1}	27.00 ± 0.71	27.24 ± 0.37^{a1}
400 mg/kg LL	48.80 ± 0.73	46.80 ± 0.63^{a2}	36.54 ± 0.18	34.36 ± 0.31^{a2}	26.80 ± 0.37	29.60 ± 1.52^{a2}
600 mg/kg LL	49.00 ± 0.56	47.10 ± 1.08^{a2}	36.54 ± 0.18	36.02 ± 0.07^{a2}	28.60 ± 0.51	31.80 ± 0.68^{a2}

Data are expressed as means ± standard error of the mean; n = 6; [a] compared to vehicle; [b] to 200 mg/kg; [c] to 25 mg/kg CQ; [1] $P < 0.05$; [2] $P < 0.01$; [3] $P < 0.001$ with respect to vehicle control.

Day 0: weight, temperature and packed cell volume pre-treatment on day zero; Day 4: post-treatment on day five; CQ, chloroquine; LL, Leaf latex; NC, negative control; PCV, packed cell volume.

The effect of the leaf latex of Aloe weloensis **in curative test**

The finding showed that parasitemia reduction was significant($p < 0.001$) at 200, 400 and 600 mg/kg doses of the latex with suppression of 36%, 58% and 64% respectively (Table 4). The result showed that all doses of the latex endowed curative effect with respect to vehicle control. Curative effect of 200 mg/kg dose was siginificantly ($p < 0.01$) lower than chloroquine ($p < 0.001$). All doses of the latex siginificantly ($p < 0.01$) improved mean survival time of the mice compared to the vehicle control. The survival time of the mice treated with 200 mg/kg dose was significantly ($p < 0.01$) lower than chloroquine.

Table 4
Parasitemia level, % Suppression and survival time of infected mice treated by the leaf latex of *A. weloensis* in curative test

Groups	% Parasitemia	% Suppression	Survival times (days)
10 ml/kg NC	76.85 ± 0.61	00.00	8.12%±0.41
25 mg/kg CQ	00.00 ± 0.00	100.00^{a3b3c2d2}	30.00 ± 0.00^{a3b2}
200 mg/kg LL	40.37 ± 0.71^{a3e2}	36.17^{a3e3}	12.72 ± 0.45^{a2e2}
400 mg/kg LL	30.25 ± 0.45^{a3e2}	57.69^{a3e2}	20.14 ± 0.62^{a2}
600 mg/kg LL	23.65 ± 0.57^{a3e1}	63.51^{a3e2}	23.87 ± 0.73^{a2}
Data are expressed as means ± standard error of the mean; n = 6; [a] compared to vehicle; [b] to 200 mg/kg; [c] to 400 mg/kg; [d] to 600 mg/kg; [e] to 25 mg/kg CQ; [1] $P < 0.05$; [2] $P < 0.01$; [3] $P < 0.001$ with respect to vehicle control.			
CQ, chloroquine; LL, Leaf latex; NC, negative control.			

Discussion

Antimalarial activity of the leaf latex of *Aloe weloensis* was evaluated *in vitro* and *in vivo* models that offer promising evidence for the development of novel antimalarial drugs from herbal medicine. The *in vitro* test was evaluated on chloroquine sensitive *P. falciparum* 3D7 strain while the *in vivo* tests were evaluated on *P. berghei* infected mice since *berghei* produce disease similar to human plasmodium infection and sensitivity to standard drug chloroquine (7, 23, 34).

In this study, the leaf latex of *Aloe weloensis* demonstrated concentration dependent antioxidant activity in stable and sensitive free radical (DPPH) (35). Qualitative detection showed that the color of test solution changed from violet to slightly yellow color. The strongest free radical inhibition (73.54%) was observed at 400 µg/ml compared to ascorbic acid having 90.13% inhibition at 400 µg/mL.50% inhibitory concentration of the latex (IC50 = 14.25 µg/ml) was found to be comparable with ascorbic acid (IC50 = 3.97 µg/ml). The current finding in line with concentration dependent antioxidant activity of latex of *Aloe schelpei* (36) and *Aloe megalacantha* (37) in DPPH assay.

In this study, the leaf latex *A. weloensis* showed potent antimalarial activity against the 3D7 strain of *P. falciparum*. Parasite inhibition was found to be concentration dependent with IC50 values of the leaf latex and chloroquine were 9.14 and 0.02 µg/mL respectively. According to a literature review by P.V.V. Satish et at (31) the leaf latex of *A.weloensis* was active (IC50, 5–50 µg/ml) against *P. falciparum* 3D7 strain. The parasite growth inhibition pertain further investigation in the 4 day-suppressive and curative model against *P. berghei* infected mice since *in vivo* models allow the possible bio activation and the likelihood of the immune system in the eradication of infection unlike *in vitro* study (7, 9, 33).

Plant extracts are considered active when reduction or percentage suppression in parasitemia is ≥ 30% or significant prolonging the survival time of treated mice compared to the vehicle control (38–40). Thus, the leaf latex of *A. weloensis* was found to be active against *P. berghei* infected mice.

In the current study, the 4-day suppressive test was conducted for the leaf latex of *Aloe weloensis* to evaluate schizontocidal activity. The result showed that percentage suppression of parasitemia significantly changed by all doses of the latex compared to the vehicle control. Effect was found to be dose dependent. Parasitemia suppression was increased with increasing doses of the latex ($p < 0.01$ at 200 mg/kg, $p < 0.001$ at 400 and 600 mg/kg) and chemosuppression of the chloroquine was found to be 100%. The finding of the study showed that the plant was able to show greater parasite suppression at the medium and higher doses, while medium at low dose. The relative variation in parasite suppression activity among doses might be due to variation in the amount of secondary metabolites contents in the leaf latex of *Aloe weloensis.* This show that *Aloe weloensis* is endowed with a very good antimalarial activity and concordant to promising antimalarial activity of *Aloe vera*, *Aloe pulcherrima* and *Aloe megalacantha* in the genus Aloe (19–21). In another studies, significant dose-dependent parasite suppression was demonstrated after administration of *Aloe macrocarpa*, *Aloe debrana* and *Aloe sinana* which indicate a promising antimalarial activity and providing the scientific evidence for the folkloric use of the plant (41, 42).

Curative test was employed in the current study to assess effect of the leaf latex in late plasmodium infection. The finding showed that curative effect of the latex was significant ($p < 0.001$) at all doses compared to vehicle with % suppression of 36% (200 mg/kg), 58% (400 mg/kg) and 64% (600 mg/kg). This confirms that the leaf latex of *A. weloensis* endow efficacy in the late stages of plasmodium infection. The relative less chemosuppresion activity (36%) at 200 mg/kg dose of the latex possibly due to less accumulative efficacy to bring high chemosuppresion. The latex at the doses of 400 mg/kg (58%) and 600 mg/kg (64%) showed greater parasite suppression implies that dose dependent curative effect of the latex. Phytoconstituents present in the latex may block parasite growth and replication. Alkaloids endowed antimalarial effect by blocking detoxification of heme and protein synthesis in *P. falciparum* (43, 44). Quinine is alkaloidal antimalarial drug

isolated from Cinchona bark. It is useful in the treatment of multidrug resistant malaria and serve as the lead compound for derivative of chloroquine (45). Phytosteroids and flavonoids showed an outstanding activity against plasmodium parasites by boosting host immunity (46).

In this study, all doses of the latex significantly ($p < 0.01$) improved mean survival time of the mice relative to the vehicle control. This finding might probably indicate that the latex suppressed *P. berghei* and reduced the overall pathologic effect of the parasite on the mice. The longest mean survival time of the mice was strongly associated with the maximum parasitemia inhibition. According to the method in detail by Basir et al the latex of *A. weloensis* leaf was active as the latex prolonged mean survival time beyond 12 days (47).

At the same times, determined packed cell volume and rectal temperature of mice were used to predict the effectiveness of the test compounds. In contrary to human, body temperature of mice was decreased while increasing parasitemia due to decrease in the metabolism of infected mice (9, 47). In this study, the doses of the latex at 400 and 600 mg/kg showed significant ($p < 0.01$) protective effect in rectal temperature of *P. berghei* infected mice, possibly due to the latex prevents some pathological processes that cause reduction in internal body temperature, augment the immune system and metabolic rate of infected mice.

Packed cell volume reduction is one feature of *P. berghei* infected mice and determined to evaluate the effectiveness of *Aloe weloensis*. In both human and mouse, escalating parasitemia causes the clearance and/or destruction of infected RBCs, clearance of uninfected RBCs, and erythropoietic suppression and dyserythropoiesis (48). Packed cell volume was monitored before infection and on day 4 after infections for groups to predict the effectiveness of the study plant. The result of the study showed that medium and high doses of the latex significantly ($p < 0.01$) prevented PCV reduction compared to the vehicle control. This effect is in line with pack cell volume protection effect of the *Aloe megalacantha* (20). However, the low dose was devoid significant prevent hemolysis of red blood cells, might be due to high parasitemia level at the low dose relative to the other doses. The prevention of packed cell volume reduction might be due to destructive antiplasmodial effect of the leaf latex against the parasitized RBCs and the causative parasite, thereby sustaining the availability of the new RBCs produced in the bone marrow (49, 50).

Significant body weight loss was measured in vehicle control given 2%TW80 in distilled water compared to mice treated with three doses of the latex and chloroquine. The body weight loss in the experimental animals due to the appetite-suppressing result in disturbing metabolism and hypoglycemia (47). The finding showed that the latex of *A. weloensis* was found to prevent *P. berghei* induced weight loss in mice.

The finding showed that the leaf latex of *Aloe weloensis* endowed prominent antimalarial activity against 3D7 strain of *P. falciparum* and *P. berghei*. Therefore, *Aloe weloensis* might serve as potential source for the development of novel plant-based antimalarial agents.

Phytochemical screening result showed that the leaf latex of *Aloe weloensis* contained secondary metabolites (flavonoids, phenols, terpenoids, glycosides and others) which showed antimalarial activity in different plants extracts through various mechanism of action (14, 22, 23, 31). Therefore, the antimalarial and antioxidant activities of *Aloe weloensis* could elicit from single or synergetic action of these metabolites.

Conclusion

The finding of the current study has confirmed antimalarial and antioxidant efficacy of the *latex* of *Aloe weloensis* leaf, supporting folk uses of the plant. The result can serve as a step towards the development of safe and effective herbal therapy against plasmodium parasites. Further studies will require for

identification, characterization and isolation of bioactive compound (s) that possess antimalarial and antioxidant activities.

Abbreviations

EPHI
Ethiopian public health institute; OECD; Organization for Economic Cooperation and Development.

Abbreviations

AW, *Aloe weloensis*; CQ, chloroquine; NC**,** negative control.

Declarations

Ethics approval and consent to participate

The study was conducted according to OECD Guidelines and the Guide for the Care and Use of Laboratory Animals. Ethical approved was obtained from the ethical review committee of college of medicine and health sciences, Wollo University.

Consent for publication

Not applicable.

Availability of data and materials

All the datasets used/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding

Not applicable

Competing interests

The authors declared that they do not have any conflict of interest.

Authors' contributions

GGA, TAM, SA and ZDK designed the study and conducted the experiment. GGA; analyzed the data, wrote the results, prepared the manuscript and submitted approved version of the manuscript.

Acknowledgments

We are thankful to Wollo University for successful completion this experimental study.

We are also grateful to Professor Sebsebe Demisew for discovery, identification and authentication of the study plant.

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51. Legends.

Figures

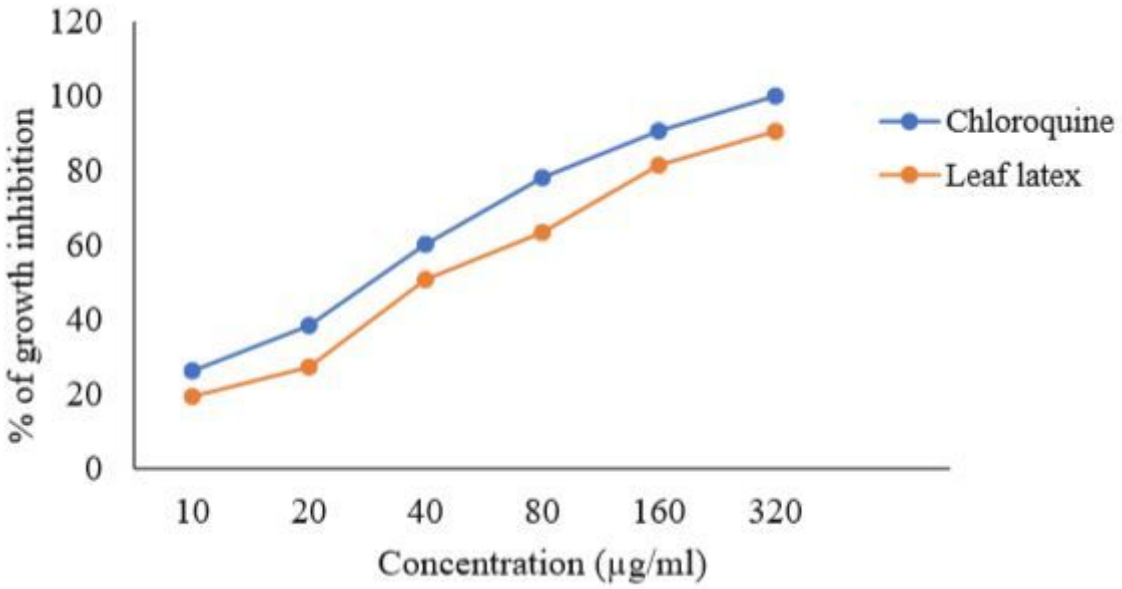

Figure 1

Growth inhibition potential of the leaf latex of A. weloensis against 3D7 strain of P. falciparum

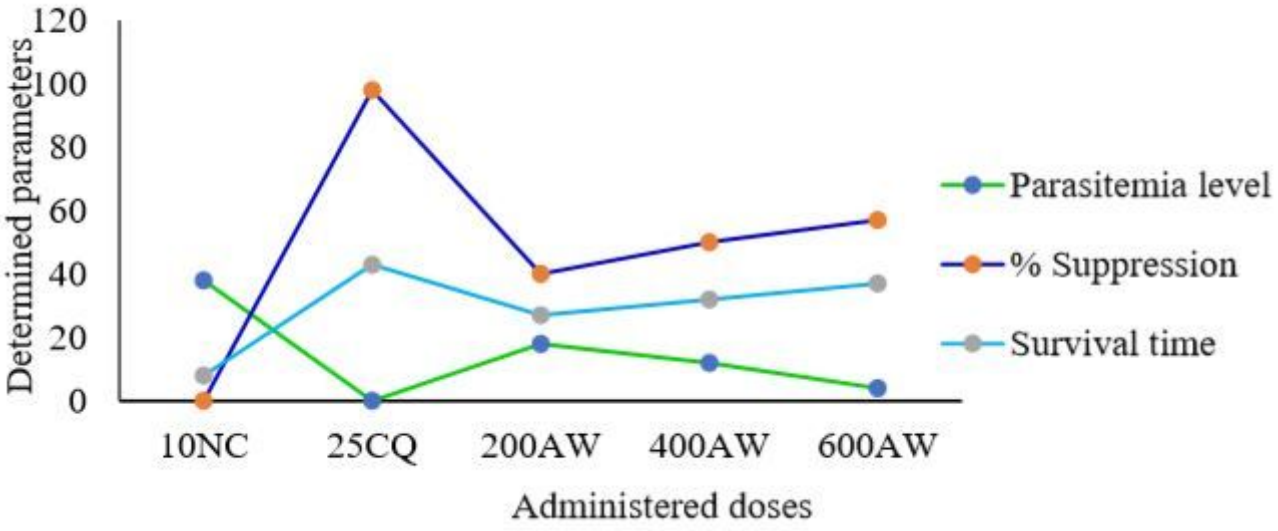

Figure 2

Parasitemia level, % suppression and survival time of the mice treated by the leaf latex of A. weloensis in the 4-day suppressive test

Supplementary Files

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