# Antimalarial activity of seed extracts of Schinus molle against plasmodium berghei in mice

**Abebe Basazn Mekuria** ( abeyohannes21@gmail.com )
University of Gondar College of Medicine and Health Sciences https://orcid.org/0000-0003-2909-2001
**Mestayet Geta**
University of Gondar
**Eshetie Melese Birru**
University of Gondar
**Desalegn Asmelashe Gelayee**
University of Gondar

**Research**

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Abebe Basazn Mekuria*, Mestayet Geta, Eshetie Melese Birru, Desalegn Asmelashe Gelayee

Department of Pharmacology, School of Pharmacy, College of Medicine and Health Sciences, University of Gondar, Chechela Street, Lideta Subcity kebele 16, P.O.box 196, Gondar, Ethiopia

**E-mail Address of Authors:**

Abebe Basazn Mekuria: abeyohannes21@gmail.com; P. O. Box, 196

Mestayet Geta: mestimengistie@gmail.com; P. O. Box, 196

Eshetie Melese Birru: meshetie21@gmail.com; P. O. Box, 196

Desalegn Asmelashe Gelayee: desefikir4ever@gmail.com; P. O. Box, 196

*Correspondence: Abebe Basazn Mekuria

Email; abeyohannes21@gmail.com

P. O. Box, 196

Tel, +251921073824

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**Abstract**

**Background:** Due to drug resistance and inefficient eradication techniques, malaria continues to be a major public health issue in countries with low- and middle-income. The seeds of *Schinus molle* are used in the Ethiopian folklore medicine for the treatment of malaria. However, this claim is not yet supported with scientific researches. Hence, the current study aims to investigate *in vivo,* antimalarial activity of hydro-alcoholic crude extract and subsequent solvent fraction of *Schinus molle* seeds on *Plasmodium berghe* infected mice.

**Methods:** A hydro-alcoholic crude extract and solvent fractions (ethyl acetate, chloroform and aqueous) of *Schinus molle* seeds were tested at different doses (100, 200 and 400 mg/kg respectively ) to evaluate *in vivo* antimalarial activity of extracts in a 4-day suppressive, curative, and prophylaxis antimalarial test models. The parasitemia level, packed cell volume, survival of date, body weight, and body temperature were used to evaluate the anti-plasmodia activity of the extracts. One way ANOVA was employed to analyze these data, followed by post hoc Tukey's HSD multiple comparison test.

**Results:** The chemo-suppressive activities produced by the highest dose (400mg/kg) of crude extract and the aqueous fraction of *Schinus molle* seeds in the four-day suppressive test were 76.03% and 73.82%($p$<0.001), respectively. In the curative test, the highest dose of crude and the aqueous fraction of *Schinus molle* seeds had 82.12% and 84.30% ($p$<0.001) suppression activity, respectively. The percentage of suppression in the prophylactic activities test of the aqueous fraction was 79.78% ($p$<0.001) at 400mg/kg compared to the negative control group. The studied plant extracts were likely anticipated to show rapid rectal temperature reduction and weight loss significantly. Among the extracts, only chloroform fraction has prevented the reduction of packed cell volume, due to the absence of saponin in the fraction. The mice which were treated with crude extract and aqueous fraction survived longer and gained net body weight as compared to vehicle-treated mice ($p$<0.001).

**Conclusion:** The crude extract and aqueous fraction of *Schinus molle* seeds possessed significant antimalarial activity. These results collectively indicate that the plant has promising anti-plasmodial activity against *Plasmodium berghei*. However, further confirmatory studies followed by isolation and characterization of the active antimalarial compound are recommended.

***Keywords:***Antimalarial activity, *Plasmodium berghei*, *Schinusmolle, Swiss albino* mice, Ethiopia

## Background

Malaria is a blood-borne protozoan infectious disease caused by *Plasmodium* species. It is one of the most pressing public health problems in low- and middle-income countries(LMICs) [1]**.** In Africa, particularly in sub-Saharan regions, it is one of the most serious medical issues with significant economic burden [2]. It is also the most prevalent infectious disease in Ethiopia (around 75%)[3], and is one of the leading causes of morbidity and mortality. Emergence and fast spread of multi-drug resistant *Plasmodium* parasites, development of insecticide resistance and absence of effective vaccine are some of the challenges in the process of aversion and control of malaria [4, 5].

In developing nations, at most times, people use traditional medicines (TM) and once in a while, it is the lone wellspring of medication for the treatment of malaria [6]. In Ethiopia, the majority of the community traditionally use herbal preparation for the treatment of malaria [7]. However, scientific investigations on crude herbal medicine preparation and isolation of active principles is very minimal [8]. *Schinus molle L* (*S.molle*) is one of the medicinal plants used traditionally for the treatment of different diseases. It is commonly known as pink pepper or American pepper and belongs to the Anacardiaceae family which is native to subtropical regions of South America [9].

In Ethiopia, *S.molle* seeds are used for the treatment of malaria by the people of Sasiga, Karsa Mojo,Mada Jalala, and Western Ethiopia. It is taken orally after the seeds are powdered and dissolved in water [9-11]. The plant is also traditionally used for the treatment of cough, tuberculosis, bronchitis, fever [12], wound around the rectal area [13], eye infection, allergy, hemorrhoids, respiratory infection [14], jaundice, diarrhea, and tonsillitis [15].

Previous pharmacological reports show that the extract of *S.molle* fruit has antimicrobial [12], immunomodulatory, antioxidant [18-20], anti-inflammatory, analgesic [21], repellent [22], larvicidal [23], antiviral, topical antiseptic as well as antifungal uses [19]. Considering the above ethno botanical and pharmacological investigations, the current study aims to evaluate *in vivo* anti-plasmodium activity of the crude extract and solvent fractions of *S. molle* seeds.

## Methods

### Plant material collection and preparation

The fresh seeds of *S.molle* were gathered from Maraki campus, University of Gondar, Gondar, Ethiopia. The plant was identified and authenticated by Dr. GetinetMersha (with voucher number AB015/2010) and deposited at the biology department, University of Gondar. The plant seeds were washed and air-dried in a shaded area at room temperature. Then, the dried seeds were coarsely powdered using an electric grinder. The powdered seeds were then placed in a plastic container and kept at room temperature until extraction.

**Chemicals and equipment**

The following chemicals and reagents were used: Giemsa stain (Science Lab, USA), trisodium citrate (Deluxe Scientific surgico, India), hydrochloric acid, potassium ferro cyanide, lead acetate, chloroform (Finkem Laboratory Reagent, India), absolute methanol (Okhla Industrial, India),isotonic saline (Addis Pharmaceuticals Factory, Ethiopia), ketamine (Rotexmedica, Germany), ethyl acetate (Research-Lab-Fine, India), acetic anhydride (Techno PharmChemical, India), and chloroquine phosphate (Addis Pharmaceuticals Factory, Ethiopia),Whatman filter paper No.1 (Whatman, England), test tubes, beakers, funnels, measuring cylinders, glass rods, spatulas, oral gavages (oral feeding syringes), syringes (1 mL, 3 mL, and 5 mL) with needles, desiccators, digital analytical balance (Mettler Toledo, Switzerland), digital thermometer, isotonic saline, micro-hematocrit reader (Hawksley and Sons, England), microscope (CHK2-F-GS, Taiwan), rotary evaporator (Buchi type TRE121,Switzerland), centrifuge (GelmaAwhksley, England), and lyophilizer (Operan, Korea vacuum limited, Korea).

**Extraction and fractionation**

One thousand gram (1kg) of dried coarsely powdered seeds of *S.molle* was macerated in hydro-methanol (80%). After 72 hours, the macerated blend was first sifted using gauze and then, filtered using filter paper no 1. The residue was re-macerated twice for seventy-two hours and filtered again. The combined extract was placed in deep freeze overnight and dried by using a lyophilizer. A portion of the methanolic crude extract was suspended in a separator funnel using distilled water as a solvent. The suspension was then shaken by adding chloroform and the chloroform fraction was collected by flask beneath. Next to this, the aqueous portion was shaken with ethyl acetate to obtain the ethyl acetate fraction. The ethyl acetate and chloroform fractions were allowed to concentrate in a rotary evaporator and the aqueous residue was lyophilized to obtain acetate, chloroform and

aqueous fraction, respectively. Finally, all the fractions were put in an amber bottle and stored in a refrigerator until use.

**Phytochemical analysis of the crude extract and solvent fraction**

The preliminary phytochemical screening test was carried out for the presence of terpenes, alkaloid, steroidal compounds, phenolic compounds, tannin, triterpenoids, sesquiterpenoid, triterpenoid, saponin, and flavonoid using standard procedures [16].

**Acute toxicity testing**

Five female Swiss albino mice were randomly selected. After having them, fast for 3 hours and a fixed dose of 2000 mg/kg of *S.molle* seeds crude extract was administered to a single mouse via oral route. Similarly, food was withheld for 1hour after extract administration. Then, the mouse was observed for gross changes such as loss of appetite, hair erection, lacrimation, tremors, convulsions, salivation, diarrhea, mortality, and other signs of toxicity for the next 24 hours. If death was not observed within24 hours, then 4 other mice were sequentially dosed with the same dose of the extract and observed daily for any signs of toxicity for 14 days [17].

***In vivo* antimalarial tests**

**Experimental animals grouping and dosing**

Six-eight weeks old, 24 to 35 gm body weight, Swiss albino mice were brought from Ethiopian Public Health Institution, Addis Ababa. The mice were kept in plastic cages with softwood shavings and presented in a 12/12 dim-to-light cycle with free access to food (pellet) and water. Before starting the experiment, the mice were acclimatized to the lab conditions for one week [18].The mice were then randomly assigned into five groups(each group having five mice). The negative control group (Group I) received 0.5 ml/kg of distilled water. The mice in groups II, III, and IV were treated with 100, 200, and 400 mg/kg of extract (both crude and solvent fraction) orally, respectively in all antimalarial testing models. Mice in the positive control group were treated with 25 mg/kg chloroquine phosphate.

**Parasite and parasite inoculation**

A chloroquine-sensitive *Plasmodium berghei* (ANKA strain), obtained from Aklilu Lemma Institute of pathobiology, was used in this study. The donor mice with 30–37 percent of parasitemia levels were selected from previously infected mice by *P.berghei*. After confirming the required level of parasitemia, the mice were anesthetized with ketamine and blood was collected via0.5 % trisodium citrate containing tube through cardiac puncture. Then, the blood was diluted with normal saline (0.9%) and each mouse was given 0.2 ml diluted blood intraperitoneally [19, 20]. The volume of normal saline required in the dilution of blood was determined by considering the percentage parasitemiaof the infected donor mice [21].

**Four-day suppressive antimalarial test (Peter's test)**

The four-day suppressive test described by Peter was used to assess the schizontocidal effect of plant extracts [22]. Twenty-five female albino mice were randomly assignedinto five groups (n = 5) and treated as explained above. The treatment was initiated at three hours after inoculating the parasite (D0) and continued daily for four consecutive days (from D0 to D3). On day 4 (D3), thin blood film was prepared from the tail of each mouse for determining the level of parasitemia.

**Curative antimalarial activity test (Rane's test)**

Determination of the remedial capability of the most active solvent fraction and the crude extract was done according to the techniques illustrated by Peters and Ryley [23]. On the first day (D0), selected mice were inoculated with standard inocula of $1 \times 10^7$ infected red blood cells intraperitoneally. After affirmation of parasitemia, on 3$^{rd}$ day (D2) the infected mice were randomly grouped into five (three tests and two control) groups and treated accordingly as described above. Then, the daily treatment was preceded for five consecutive days (up to D7). On day seven, 10% of Giemsa stained thin blood film was prepared from the tail of each mouse and then their parasetemia level was determined.

**Prophylactic antimalarial test (Repository test)**

Assessment of the repository ability of extract was conducted according to the techniques described by Fidock et al [19]. Grouping and dosing of mice were made as described above. The dosing of

mice was started on the first day (D0) and continued up to 4$^{th}$ day (D3). On day D4, each mouse had received 0.2 ml of blood infected with $2 \times 10^7$ *P.berghei* intraperitoneally. After seventy-two hours of inoculation (D7), a thin blood smear was prepared from the tail of each mouse and then the level of parasitemia was determined.

**Parasitemia level measurement**

Thin smears were prepared by using 76 × 26 mm slides from each mouse tail. Then, fixed and stained by using absolute alcohol and 10% Giemsa blue for 15 minutes respectively. After staining, the slides were gently washed using demineralized water and allowed to air dry. Finally, the air-dried slides were observed under an oil immersion microscope with one hundred times of magnificent nosepiece. After counting six fields from each slide, the percentage of parasitemia was calculated [25] using the following equation [20].

$$\% \text{ suppression} = \frac{\text{mean parasitemia of untreated group} - \text{mean parasitemia of treated group}}{\text{mean parasitemia of untreated control groups}} \text{ x } 100$$

**Packed cell volume measurement**

Packed cell volume (PCV) was used to estimate the effectiveness of the test extracts in anticipating hemolysis resulting from the invading parasitemia. Heparinized capillary tubes were used to collect blood from each mouse tail .The tubes were then filled up to 3/4$^{th}$ of total volume and sealed with dry sealing clay. The sealed tubes were allowed to centrifuge in micro-hematocrit centrifuge with a speed of 12,000 revolutions per minute. After five minutes, the sealed tubes were taken out from the micro-hematocrit centrifuge and the percentage of PCV was determined using a calibrated micro-hematocrit reader [24].

**Determination of mean survival time**

Similar to other parameters, mean survival time is also used to assess the antimalarial efficacy of plant extracts. For an extract to be considered as an active antimalarial agent, mice in the tested groups should have greater mean survival time than the vehicle-treated group. In all the above models, each mouse was observed from parasite inoculation (D0) to 30 days (D29) for determining survival time.

The mortality of each group of mice was recorded and mean survival time (MST) of each group was calculated using the following mathematical equation [20].

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

**Monitoring of body weight and temperature changes**

Changes in body weight and rectal temperature are commonly used to evaluate the efficacy of extracts against malarial infection [19]. In the four days suppressive test, the body weight was taken on the first and fourth day. In the case of curative test, body weight and rectal temperature of each mouse were taken before parasitemia inoculation (on D0) and after inoculation at day seven. In the prophylactic test, the body weight and rectal temperature of the mouse in each group were taken before treatment (D1) and after treatment (D7).

**Anesthesia and euthanasia**

Having completed the experiment, the mice were killed using inhaled $CO_2$as per the recommendation of the guideline of the American Association for Laboratory Animal Science [25].

**Data analysis**

The raw data on the levels of parasitemia, changes in body weight, variations in rectal temperature and survival times were analyzed by using SPSS version 21.0software. All data were presented in the form of mean ± SEM (standard error of the mean). One-way ANOVA followed by Tukey's HSD multiple comparison tests were used to analyze and compare the mean difference between values of the measured parameters. A *p*-value of less than 0.05 was considered statistically significant.

**Ethical approval**

In laboratory procedures, all mice were handled and cared according to the internationally accepted guidelines [26]. Ethical clearance was requested and obtained with approval number SoP/45/10 from the department of Pharmacology animal Ethics Committee, College of Medicine and Health Sciences, University of Gondar.

## Results

### Extraction yield

The percentage yield of the crude extract was 17.2%, and had a deep red color. The yields of the dried solvent fractions were 11.6gm (29.1%) chloroform, 4.8gm (12%) ethyl acetate and 23.6gm (57.5%) aqueous.

### Acute toxicity study

The acute oral toxicity test showed that there was no visible sign of gross physical, behavioral changes or resulting death at 2gm/kg/dose of the extract within the first twenty-four hours and the following fourteen days. This suggested that the lethal dose (LD50) of *S.molle* seeds extract is greater than 2000mg/kg bodyweight of the animal.

### *In vivo* antimalarial activity test

### Effect on four days suppressive test

### The effect of crude extract and solvent fraction

The crude extract of *S.molle* seeds was demonstrated at doses of 100, 200 and 400 mg/kg and showed 39.08%, 57.14%, and 76.03% of chemo-suppression, respectively. The chemo-suppression analysis revealed that the extract had dose-dependent inhibition ($p<0.001$) of the parasitemia compared to the vehicle-treated group. The extract also increased the survival time of mice significantly ($p<0.01$ for SM100, $p<0.001$ for SM200 and SM400) in contrast to the negative control (NC). The extract treated mice survived for 9.10 ± 0.37, 11.40 ± 0.51 and 15.40 ± 0.51 days at doses of 100, 200 and 400 mg/kg bodyweight, respectively. Mice treated with SM400 exhibited significantly ($p<0.001$) decreased parasitemia level and increased survival time than groups of mice treated with SM100 and SM200 (Table 1).

The mean comparison analysis indicated that all doses (SM100, SM200 and SM400) of crude extract prevented weight loss significantly at $p<0.05$, $<0.001$ and $<0.001$ respectively compared to vehicle-treated mice. The maximum prevention of weight loss related to the elevation of parasite

level was shown at dose of 400 mg/kg. The mice treated with the middle and the largest doses of crude extracts significantly ($p$<0.001) increased body weight compared to the NC group. There was no detectable difference in preventing PCV associated with parasitemia levels between all doses of crude extracts and NC groups. Analysis of rectal temperature revealed that the crude extract of *S.molle* seeds prevented the reduction of rectal temperature significantly ($p$<0.05 for SM200, $p$<0.001 for SM200 and SM400) in a dose-dependent manner as compared to the NC group. Both SM200 and SM400 crude extracts had a comparable attenuation effect on the reduction of rectal temperature compared to the positive control group (CQ25) (Table 2).

Similar to the crude extract, all fractions (except chloroform 100mg/kg) showed significant reduction of parasitemia level ($p$<0.001 in all cases except ethyl acetate 100mg/kg fraction $p$<0.01) compared to vehicle-treated mice. Correspondingly, the rank order of parasitemia suppression of solvent fractions was aqueous (84.30%) > ethyl acetate (73.99%) >chloroform (69.81%). Survival date was significantly prolonged ($p$<0.001) by all doses of fractions (except low dose of chloroform and ethyl acetate) compared to the distilled water treated group (Table 1).

All types and doses of the fraction (except low dose of chloroform) prevented bodyweight reduction significantly ($p$<0.001) as compared to NC mice. With regard to rectal temperature, all solvent fractions (except chloroform fraction at 100mg/kg) significantly prevented the reduction in rectal temperature ($p$<0.001) compared to mice treated with vehicle. PCV reduction was attenuated dose-dependently by 200mg/kg and 400mg/kg doses of the chloroform fraction, but no detectable changes were noted with the other two fractions compared to the NC group (Table 2).

**Effect on curative test**

**Crude extracts and solvent fractions**

The crude extract decreased parasitemia level by 38.42%, 55.10% and 73.49% for SM100, SM200 and, SM400, respectively compared to NC mice. Similarly, the aqueous fraction which had the highest antimalarial activity in Peter's test was further assessed for its effectiveness on the curative test. All doses of aqueous fraction showed a dose-dependent reduction ($p$<0.001) of parasitemia levels compared to NC mice. The maximum inhibition (82.12%) of parasitemia levels was attained using 400 mg/kg dose of the aqueous fraction. The parasitemia inhibition seen with CQ25 was

significantly ($p$<0.001) higher than the crude extract and aqueous fraction. Mean survival time analysis revealed that mice that received all doses of crude (except SM100 ($p$<0.05) and aqueous fraction lived longer (with significant value $p$<0.001) than mice in the NC group. The mice treated with the largest dose (400 mg/kg) of crude extract and aqueous fraction lived longer (14.00±0.68 days for crude extract and 15.80±0.20 day for aqueous extract) than those in the NC group. The standard-drug treated group lived longer (30±00) days which is significantly ($p$<0.001) higher compared to all tested and NC groups (Table 3).

In the curative test, all doses of crude extracts and aqueous fractions exhibited significant ($p$<0.001) protection against body weight loss compared to the NC group. Similar to the 4-day suppression test, mice treated with the largest doses of crude extract and all doses of the aqueous fraction (except AF100 mg/kg) showed significant dose-dependent body weight gain compared to the NC group. The rectal temperature analysis revealed that all doses of crude and aqueous fractions attenuated the reduction of rectal temperature significantly ($p$<0.001) compared to the NC mice. However, there was no significant difference compared to CQ25. The highest elevation of rectal temperature was shown in mice received the largest dose of the aqueous fraction (1.01%) next to CQ25 (1.28%). Like Peter's test, the crude and aqueous fraction didn't show a significant protective effect on PCV reduction compared to NC mice (Table4).

**Effect on prophylactic test**

The aqueous fraction of *S.molle* seeds showed significant ($p$<0.001) reduction of parasitemia level compared to the NC mice. It showed that dose-dependent reduction of parasitemia level by 54.84%, 69.76%and 79.78% for AF100, AF200 and AF400, respectively compared to the NC mice. All doses of the aqueous fraction increased survival date significantly compared to NC mice. *S.molle* extract-treated mice lived for 9.20 ± 0.37, 10.80 ± 0.37 and 13.20 ± 0.58 days at dose of 100, 200 and, 400 mg/kg per day, respectively (Table 5).

As shown in Table 6, the aqueous fraction ablated body-weight reduction significantly by$p$<0.05, $p$<0.01 and $p$<0.001 at AF100, AF200 and AF400 doses, respectively as compared to the NC group. A comparison of extract and CQ25 treated groups did not show a significant difference in body weight. Regarding rectal temperature, the aqueous fraction of *S.molle* seeds significantly ($p$<0.001) attenuated a rapid reduction in rectal temperature as compared to NC. Similar to the suppression and curative test, in prophylaxis test the PCV reduction was not significantly prevented by all doses of aqueous fractions as compared to NC.

**Phytochemical screening**

Preliminary phytochemical study of the hydro-methanolic crude extract of seeds of *S.molle* revealed the presence of alkaloids, saponins, phenolic compounds, cardiac glycosides, tannins, terpenoids, flavonoids, triterpenoid, and anthraquinones. However, diterpenoid and steroids were absent from the crude extract. The phytochemical composition of the solvent fractions is depicted in table 7. The aqueous fraction appeared to be rich in secondary metabolites as compared to the other two fractions.

## Discussion

The *in vivo* model was used for this study owing to the possible pro-drug effect and involvement of the immune system in killing the parasites [6]. In this study, the antimalarial activity screening was conducted by using chloroquine-sensitive *p.berghei* used for the identification of several antimalarial medications like mefloquine, chloroquine, halofantrine, and artemisinin derivatives. In all anti-plasmodial test models, the determination of percent inhibition of parasitemia level is the gold standard parameter [20].

In Peter's antimalarial test model, the crude extracts of seeds of *S.molle* have reduced the parasitemia level by 76.3%. The extract to be considered as active ought to decrease the parasitemia level at least by 30% [27, 28]. Therefore, the extract of *S.molle* seeds is considered as active and endowed the potential antimalarial activity. This strong schizontocidal effect may be related to secondary metabolites that are present in its seeds. The seeds contained alkaloids, flavonoid, phenolic compounds and terpenoids which might be contributed to this antimalarial activity. Among those secondary metabolites, alkaloids have critical importance and fascinating antimalarial effects [29, 30]. In addition to this, *S.molle* had immunomodulatory, antioxidant [31], anti-inflammatory and analgesic effects [32] that might contribute to its antimalarial activity. In addition to this, the *S.molle* seeds extract showed a dose-dependent chemo-suppressive effect that could be attributed to the concentration of bioactive principles in doses delivered to experimental animals. This type of dose-dependent antimalarial activity was observed with other medicinal plants[33].Among the fractions, the aqueous fraction was found to possess the highest blood schizontocidal activity than ethyl acetate and chloroform fraction. A similar finding was reported the polar solvent (aqueous extraction and methanol) had better chemo-suppressive activity than the other non polar solvent (chloroform) [34]. This might be explained by the possible localization of the most active components in aqueous fraction than ethyl acetate and chloroform fraction of *S.molle* seeds.

In the Rane's test, blood smears were prepared and the parasitemia level was determined each day to assess the remedial capacity of tested extract. Both crude extract and the aqueous fraction of *S.molle* seeds displayed a significant ($p$<0.001) curative effect on *P. berghei* infected mice. Then again, the comparison between crude and the aqueous fraction, the aqueous fraction produced better chemo-suppressive activity than the crude extract. This finding was in line with the crude extract of *S.guineense* [35] and the aqueous fraction of *Croton macrostachys* extract [36]. The result sections showed that all doses of *S.molle* seeds extracts began to decrease parasitemia levels after the second dose of extract. This might be suggesting that the plant extract has delayed onset of action. Similar to the 4 days suppressive and curative tests, the aqueous fraction also had dose-dependent high chemo-prophylactic activity. In the repository test, the aqueous fraction showed that a relatively low percentage of reduction of parasitemia levels as compared to activity on Peter's and curative test. This might be related to rapid liver metabolism and clearance of the active ingredient.

An effective anti-plasmodial active agent derived from medicinal plant extract has relied on anticipated bodyweight reduction, rectal temperature reduction and anemia which are the common features of malaria-infected mice [19]. All doses and types of extraction (crude and solvents) of *S.molle* seeds ablated bodyweight reduction significantly. This finding is similar to the study done on crude and solvent fraction of *A. schimperi* [34] and *C.macrostachyus* [37]. Significant bodyweight gain was also observed among mice treated with the highest dose of crude extract and aqueous fraction. This result is in line with other studies done on plant extract like seeds of *B.nigra* [38] and leaves of *B.rotundifolia* [39]. This may be related to the nutrient content, appetite-stimulating activity or possession of other pharmacologic activities of *S.molle* seeds like immunomodulatory [32], analgesic [33] and antipyretics activity [12].

A decline in metabolism rate in parasite-infected mice happened before death and is accompanied by a corresponding decrease in internal body temperature [19]. Ideally, active compounds should have to prevent rapid dropping of the rectal temperature as parasite level escalates. In this investigation, a significant dose-dependent attenuation of the rectal temperature reduction effect was seen in all tested models. This finding is strengthened by other medicinal plants' effects on rectal temperature such as leaves of *B.rotundifolia* [39]. This might be associated with the quick reduction of parasitemia levels by the study plant extracts. Among solvent fraction, the aqueous fraction had the highest preventive activity against rectal temperature decrement than other extracts. This might be due to the possible localization of the responsible active ingredient for this effect into the aqueous fraction. In the curative test, active constituents ought to attenuate the rapid dropping of body temperature from baseline and return to normal temperature range [19]. The increase in rectal temperature was observed by all doses of crude extract (except lowest dose, SM100) and aqueous fraction in the curative antimalarial model. This may be due to the extracts had a high level of reduction of parasitemia levels as well as appetite-stimulating activity [28].

As indicated by Lamikanra et al, the PCV parameter is estimated to assess the viability of the extracts in anticipating hemolysis because of the spread of the parasite in RBCs [40]. The pathogenesis of malaria causes severe anemia due to hemolysis of RBCs, distraction normal RBCs, erythropoietin inhibition and dyserythropoieticeither by malaria reproduction or by reticuloendothelial cells action [41]. In addition to this, the malaria parasites cause certain cells to produce free radical like reactive oxygen species thereby resulting in hemoglobin degradation [42]. Along these lines, a perfect anti-plasmodium agent got from plants is relied upon anticipation of the decrement of PCV. In this study, one could note that the chloroform was the only fraction that prevented PCV reduction significantly. This finding corroborates with other studies conducted on chloroform fraction of various plants such as *C.macrostachys* leaves [37] and *D.Angustifolia* [43]. Failure of the crude extract, aqueous and ethyl acetate fraction to reverse PCV reduction might be related to the presence of saponin, which is known to have strong hemolytic effects [44].

The effectiveness of the anti-plasmodial activity of medicinal plant extract was also measured by the mean survival time of treated mice infected with *P. berghei* [45]. In our study, all doses of crude and solvent fraction prolonged MST significantly but much shorter than standard drug. This finding corroborates with a study done on *N.sativa* [46]. This may be related to the shorter duration of action due to the rapid elimination of plant extracts from the body. Moreover, the aqueous fraction prolonged MST more than crude and other solvent fractions in all the three antimalarial activity test models. However, no significant difference was detected between SM400 and AF200 in the mean survival date. The extract had MST longer than 12 days and is considered as an active antimalarial agent [47] which further affirms the potential antimalarial activity of the extract. According to Daharo et al[45]classification of antimalarial activity, the crude extract of *S.molle* seeds has a good antimalarial effect, while the aqueous fraction has very good antimalarial.

## Conclusion

Both the crude extract and solvent fractions of *S.molle* showed significant antiplasmodial activity in a dose-dependent manner. The aqueous fraction demonstrated the highest antimalarial activity. The extracts also attenuated body weight loss and the rapid dropping of temperature. However, except chloroform fraction, none of the extracts prevented the reduction of PCV significantly. Moreover, both crude and solvent fractions prolonged the mean survival time of mice compared to the negative control in all the three antimalarial activity test models. Generally, the crude and solvent fractions of the plant have good antimalarial activity and which may partly justify the claim by traditional practitioners about the use of this plant against malaria. However, further evaluation is needed to isolate, identify and characterize the active ingredients responsible for the observed antimalarial activity of the plants as well as chronic toxicity evaluation of the studied plant.

## Abbreviations

ANOVA: Analysis of Variance; CQ: Chloroquine; D: day; DW: Distilled Water; EHNRI: Ethiopian Health and Nutrition Research Institute; FAS: Fatty acid biosynthesis; LD50: Lethal dose 50 (Median lethal dose);MST: mean survival time; NC: negative control; OECD: Organization for Economic Co-operation and Development; P.*berghei: Plasmodium berghei;* PCV: Packed Cell Volume; RBC: Red blood cell; SEM: Standard error of the mean; SM: *Schinus Molle;* TM: Traditional Medicine.

## Declarations

### Ethical approval and consent to participate

The ethical clearance to experiment was obtained from the animal ethics committee of the Department of Pharmacology, School of Pharmacy, University of Gondar.

### Consent for publication

Not applicable.

### Availability of data and materials

The data sets supporting the finding of this study can be obtained from the corresponding author upon a request.

### Competing interests

The authors declare that they have no conflict of interest

### Funding

Not applicable.

### Authors' contributions

ABM contributed to designing the study, performed the experiment and write up of the final research, and manuscript preparation and finalization. MG and DAG participated in data analysis, data interpretation and supervision of the study. EMB contributed to experimenting, write up of the final research and manuscript preparation. All authors read and approved the final manuscript.

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