Evaluation of antimalarial activity of a traditionally used medicinal plant in Ethiopia against *Plasmodium berghei* in Swiss Albino Mice

Sibhatu Gebrehiwot[1]*, Mirutse Giday,[2] Berhanu Erko[2], Yalemtsehay Mekonnen[3]

[1]*(Department of Biology, College of Natural and Computational Science, Raya University, Ethiopia)*
[2]*(Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia)*
[3]*(Department of Biomedical Sciences, College of Life Science; Addis Ababa University, Ethiopia)*
Corresponding author: Sibhatu Gebrehiwot

Abstract: *The objective of the present study was to investigate the antimalarial activities of root extracts of Carissa spinarum Linn. The plant materials were collected from its natural habitat and extracted using 80% methanol and non-polar solvents. A rodent malaria parasite, Plasmodium berghei, which was maintained at the Aklilu Lemma Institute of Pathobiology laboratory, was inoculated into Swiss albino mice. The mice were infected with $1x10^7$ parasites intraperitoneally. The extracts were administered by standard intra gastric tube daily for four days starting from the day of parasite inoculation. The control groups were given the same amount of solvent (vehicle) used to suspend each dose of the extract. Chloroquine was used as a standard drug and was administered through the same route. Data obtained from the experiment was analyzed using one way ANOVA. The results indicated that the root plant extracts exhibited significant antimalarial activities. The hydro-alcoholic and chloroform extracts of C. spinarum significantly (P<0.05) inhibited parasitaemia in a dose dependent manner but only the higher doses of the hydro-alcoholic extract prevented Packed cell volume (PCV) fall due to parasite infection (P<0.05). In addition, the higher doses increased the survival time of the infected mice and prevented body weight loss. The highest suppression was shown in hydro-alcoholic extracts with 62.88% parasite suppression at the dose of 1000 mg/kg. In addition, the plant extracts treated mice did not exhibit any signs of acute toxicity up to dose of 2000 mg/kg.*
Therefore, the result reveals the potential use of this medicinal plant in the folk medicine of Ethiopia as antimalarial.
Key words: *Malaria, Carissa spinarum, traditional medicine, Plasmodium berghei, in vivo*

I.   Introduction

Malaria is one of the world's leading health problems, causing about 429,000 deaths in 2015, the vast majority of deaths (99%) were due to *Plasmodium falciparum* malaria[1]. In the aforementioned year, most of the deaths (approximately 92%) were estimated to have been occurred in the sub-Saharan Africa region. Most of the victims were found to be children who are particularly affected by the disease with 70% of malaria caused deaths occurring among the under five-year age group[1, 2]. In Ethiopia, the majority (around 68%) of population lives in malarious areas or potentially malarious [3]. Although improvements have been made in malaria control strategies, the disease is still major public health problem and a leading cause of outpatient consultations, admissions and death in the country[4]. Ethiopia is a home of diversified flora which have been widely using as traditional medicine [5]. Ethiopians have used traditional medicines for many centuries, the use of which has become an integral part of the different cultures in Ethiopia[6]. On the other hand, 90% of the population in Ethiopia uses traditional medicine for primary health care[7]. Even though these plants are used extensively, divergent preparation and use patterns of antimalarial herbal remedies, as well as associated toxicity risks and countermeasures, generally demand deeper, exhaustive investigations[8]. *C. spinarum* is one of the medicinal plants of Ethiopia[9] and has been reported to have many medicinal uses. Root decoction of the plant is used to treat malaria[10]. The ethanolic showed significant hepatoactivities against CCl_4 and paracetamol-induced hepatotoxicity[11]. Aqueous extracts of roots of *C. spinarum* have exhibited significant activity against Herpes simplex virus (HSV) *in vitro* and *in vivo* for both wild type and resistance strains of HSV [11]. Therefore, this study was designed to validate the scientific relevance of the claimed medicinal plant, that is, root extract of *C. spinarum* as antimalarial treatments.

II. Materials and methods

Plant materials collection

The plant materials used in the present study were collected from its natural habitat. The selection of plants was done on the basis of traditional reputation of particular plants for efficacy in the treatment and management of malaria as used by the local communities' at large and traditional healers in particular. The plant was identified and authenticated by a botanist, at Addis Ababa University, Aklilu Lemma Institute of Pathobiology and a voucher specimen of plant samples were deposited at the National Herbarium of Addis Ababa University.

Preparation of crude plant extracts

The plant materials were cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered using a grinding mill (IEC,158 VDE 066, Germany). The powdered plant materials were packed in plastic bag and kept until extraction. The coarsely powdered plant materials were weighed by sensitive digital weighing balance (Scientech balance) and repeatedly extracted in hydro-alcoholic and alcoholic solvents in maceration flasks (Erlenmeyer flask). The powdered plant materials were soaked separately in alcoholic (non-polar) and 80% methanol (hydro-alcoholic) for 72 hours by shaking using an orbital shaker at 130 rpm. After 72 hours, the extract was separated from the marc by filtration (Whatman filter paper number 1 with pore size 0.7μm). This procedure was repeated three times. In the non-polar extracts, the solvents were removed by evaporation under reduced pressure by rotary evaporator (Buchi Rota vapor, TRE 121, Switzerland) in distillation flask at 45 rpm and temperature 45^0C to obtain the crude extracts of each plant. The extract was further concentrated to dryness in a water bath. While the hydro-alcoholic extracts filtrate was frozen in refrigerator overnight and then it was further frozen and dried in a lyophilizer (CHRIST, 3660 Osterode/harz/ France) at -40^oC and vacuum pressure to obtain a freeze dried product. Lastly, the semi-solid crude extracts were then stored in a refrigerator at 4^oC in air tight bottle containers until used for the experiment.

Experimental Animals

Swiss albino mice (25-38 grams), 6-8 weeks of age obtained from Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University, were used for the study. They were given a standard diet and tap water *ad libitum*. For *in vivo* anti-malarial assays of plant extracts, chloroquine sensitive strain of *P. berghei* maintained in mouse at the ALIPB, Addis Ababa University were used.

Parasite Inoculation

Plasmodium berghei was obtained from Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University. Albino mice previously infected with *P. berghei* having variable parasitaemia were used as donor. For each experiment about 1 ml *P. berghei* infected blood sample was collected by heart puncture; ethyl ether was used as anesthesia while sacrificing the donor mouse with rising parasitaemia of about 20-30% in such a way that 1 ml blood contains $5x10^7$ infected erythrocytes [12]. This was prepared by determining the percentage of parasitaemia and diluting 1ml of blood in 4 ml of physiological saline (0.9% NaCl). Each mouse was given intraperitoneally 0.2 ml of this diluted blood which contains $1x10^7$ *P. berghei* infected erythrocytes. To avoid variability in parasitaemia, the blood collected from all donor mice was pooled together. The parasites were maintained by serial passage of blood from infected mice to non-infected ones on weekly basis.

***In vivo* Toxicity Test of the Crude Plant Extracts**

The crude extracts of the root of the claimed medicinal plant intended for the anti-malarial test against *P. berghei* were evaluated for their toxicity in non-infected male Swiss albino mice aged 6-8 weeks and weighing 23-35 grams as per the guideline of OECD[14]. The mice were housed in cages and randomly selected ones were marked on the tail for individual identification. All mice were maintained on a 12-h light/dark cycle at room temperature. They were allowed to acclimatize to laboratory conditions for a week before starting the experiment. Drinking water and food were provided *ad libitum* throughout the experiment, except for the short fasting period where the drinking water was still in free access but no food supply was provided 12 h prior to treatment[15]. For the test of each plant extract, a total of 20 mice were selected and randomly divided into four groups of five mice per cage; one control group and three test groups. 0.2ml of hydro-alcoholic extracts of the selected medicinal plants were given orally in a single dose of 2000, 3000 and 5000mg/kg for the acute toxicity. The mice in the control group received 0.2 ml of vehicle of the extract (dH20). Then, the mice were observed continuously for 1 hour, intermittently for 4 hours and a period of 24 hours for gross behavioral changes such as rigidity, sleep, mortality and other signs of acute toxicity manifestations and followed for 14 days[15].

Evaluation of suppressive activities of the plant extracts

In screening of the plant extracts, a standard Peter's four-day suppressive test was employed against CQ sensitive *P. berghei* ANKA strain in mice[16]. Male Swiss albino mice weighing 25-38 were infected with 10^7 *P. berghei* and randomly divided into five groups of five mice per cage. The infected mice were randomly divided into three test groups and two controlled groups in case of hydro-alcoholic (both in lower and higher doses) and chloroform extract of *C. spinarum* (each for chloroquine as a standard drug and dH_2O/20% DEMSO as a negative control). The test extracts were prepared in six different doses 100 mg/kg, 200 mg/kg, 400 mg/kg, 600 mg/kg, 800 mg/kg, 1000 mg/kg and Chloroquine at 10 mg/kg in a volume of 0.2 ml and vehicles at 0.2 ml/mouse. Each extract was administered as a single dose per day. In case of chloroform extract of *Carissa spinarum* only three doses were used i.e. 200, 400 and 800mg/kg of body weight and negative control were given 20% DEMSO as a vehicle. All the extracts and the drug were given through intragastric route using standard intragastric tube (gavage) to insure safe ingestion of the extracts and the drug[14]. Treatment were started after 3 hours of infection on day 0 and continued daily for four days (i.e. from day 0 to day 3). On the fifth day (Day-4), 24 h after the last dose blood sample were collected from tail snip of each mouse [17]. Thin smears were prepared fixed with methanol for 30 seconds and stained with 10% Giemsa solution for 25 minutes. Each stained slide, mainly the tail part of the smear, was examined under the microscope with an oil immersion objective of 100x magnification power to evaluate the percent suppression of each extract with respect to the control groups. The microscope had an Ehrlich's eyepiece showing about 100 red blood cells per field[18]. The parasitaemia level was determined by counting minimum of ten fields per slide with 100 RBC in random field of the microscope. Percent parasitaemia and percentage suppression was calculated using the following formula[19].

$$\% \ Suppression = \frac{Parasitaemia \ in \ negative \ control - parasitaemia \ in \ treated \ group}{parasitaemia \ in \ negative \ control} \ X \ 100$$

Determination of Packed cell Volume

The packed cell volume (PCV) of each mouse was measured before infection and on day 4 after infection. For this purpose, blood was collected from tail of each mouse in heparinized microhaematocrit capillary tubes up to 3/4th of their length. The tubes were sealed by crystal seal and placed in a microhematocrit centrifuge (Hettich haematokrit) with the sealed ends out wards. The blood was centrifuged at 12,000 rpm for 5 minutes. The volume of the total blood and the volume of erythrocytes were measured and PCV was calculated as:

$$PCV = \frac{Volume \ of \ erythrocytes \ in \ a \ given \ volume \ of \ blood}{Total \ blood \ volume} \ X \ 100$$

Determination of Mean Survival Time

Mortality was monitored daily and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse in the treatment and control groups throughout the follow up period. The mean survival time (MST) for each group was calculated using the following formula[20].

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

Determination of Body weight Change

The body weight of each mice in all the groups was measured before infection (day 0) and on day-4 in case of treatment, in the same fashion in case of sub-acute toxicity, it was measured before and after the different doses were given by a sensitive digital weighing balance (Scientech balance).

$$Mean \ body \ weight = \frac{Total \ weight \ of \ mice \ in \ a \ group}{Total \ number \ of \ mice \ in \ that \ group}$$

Data analysis

Results were presented as a mean plus or minus standard error of the mean (M ± SEM). Statistical significance was determined by one way analysis of variance (ANOVA) using SPSS version 20 for windows software. The data obtained from sub-acute toxicity, suppressive studies, mean PCV and body weight before and after infection were analyzed among different groups corresponding to each dose levels and vehicle control group at fixed time and overtime (D0 and D4). Mean PCV and body weight before and after infection and treatment were compared by two-tailed paired t-test. To observe any significance differences in the parameters across the two time periods, the average of both parameters was calculated and compared using one way

ANOVA followed by Turkey-multiple comparison test. The result was considered statistically significant at 95% confidence level (P-value <0.05).

III. Results

Acute toxicity test for the plant materials

In the present study, *in vivo* studies on the toxicological effect of hydro-alcoholic extracts from *Carissa spinarum* was carried out in test mice. Before the experiment was commenced, the mice were fasted overnight [14]. The amounts of the *C. spinarum* hydro-alcoholic extracts for acute toxicity given were 2000, 3000 and 5000 mg/kg body weight while the negative control group were given dH_2O. At the level of 2000, 3000 and 5000 $mgkg^{-1}$ bwt no major signs of acute toxicity were observed in the extract treated mice activities.

Evaluation of the Antimalarial Activity of Plant Extracts in mice
Effect of Crude Extracts on Body weight and PCV

Table-no1 shows the effect of the crude extract (lower doses) on body weight and Packed Cell Volume (PCV) of the *Plasmodium berghie* infected mice. Up on the treatment with crude hydro-alcoholic extracts (100, 200 and 400mg/kg) of the roots of *C. spinarum* did not prevent reduction in PCV due to parasitaemia but prevented body weight loss. The result showed a significant (P<0.05) reduction in PCV between days 0 and 4 in both negative controls and extract treated groups of mice but there was no significant change in body weight (P>0.05).

Table no 1: Effect of crude hydro-alcoholic root extracts (lower doses) of *C. spinarum* on body weight and PCV of *P. berghei* infected mice.

Dose mg/kg extract	PCV			Body weight		
	Day-0	**Day-4**	**Mean% change**	**Day-0**	**Day-4**	**Mean% change**
NC	52.8±0.48	40.58±0.64*	-23.14±1.45[a]	37.1±6.4	33.98±1.46	-8.4±3.32[a]
100	56.94±0.5	46.36±0.69 *	-18.58±1.58[a]	29.38±2.39	27.36±1.44	-6.8±1.54[a]
200	54.14±1.56	42.30±0.87*	-21.86±2.83[a]	33.7±8.76	30.23±6.9	-10.23±1.8[a]
400	51.68±0.74	43.23±0.66*	-16.35±2.9[a]	28.66±2.55	26.7±0.51	-6.87±1.47[a]
QC(PC)	52.17±0.74	51.82±0.72	-0.67±1.65[b]	30.34±2.3	29.46±2.5	-2.9±0.8[a]

*=there was significant change between day-0 and day-4 (P<0.05)
Means in a column followed by the same letter do not differ significantly (P>0.05)
Key=Values are presented as M±SEM; n=5; CQ= Chloroquine Phosphate; PC= Positive control; NC = negative control (0.2ml of dH2O); PCV=Packed cell volume

Table no 2 shows the effect of the crude extract (600mg; 800mg and 1000mg) on body weight and PCV of the *Plasmodium berghei* infected mice. The result following the treatment of extract of the roots of *C. spinarum*, showed that weight loss was prevented in dose dependent manner. Furthermore, these three doses also significantly prevented the reduction in PCV due to parasitaemia (P<0.05).

Table no2: Effect of higher doses of crude root extracts of *Carissa spinarum* on body weight and PCV of *P. berghei* infected mice

Dose mg/kg extract	PCV			Body weight		
	Day-0	Day-4	Mean % change	Day-0	Day-4	Mean % change
NC	52.56±1.56	41.24±3.21*	-21.53±1.6[a]	32.4±1.31	28.29±2.51*	-12.68±2.99[a]
600	51.78±2.51	46.90±3.57	-9.42±2.19[b]	31.92±3.21	28.74±2.17	-9.96±102[a]
800	50.19±1.43	47.72±1.73	-4.92±0.9[b]	27.2±2.37	31.8±3.23	16.9±7.6[b]
1000	51.82±2.15	51.64±2.13	-.03±0.11[b]	30.6±1.23	30.16±1.21	-1.43±5.8[ab]
QC(PC)	53.37±0.27	52.56±0.23	-1.51±0.54[b]	35.8±3.15	34.97±1.57	-2.31±2.4[a]

*=there was significant change between day-0 and day-4(P<0.05)
Means in a column followed by the same letter do not differ significantly (P>0.05)
Key=Values are presented as M±SEM; n=5; CQ=Chloroquine Phosphate; PC=Positive control; NC=negative control (0.2ml of dH2O)

Table no 3 shows effect of chloroform extract of *C. spinarum* on pcv and body weight of *p. berghei* infected mice. Tthe chloroform extract of *C. spinarum* prevented both body weight loss and PCV reduction due to parasitaemia infection.The result showed that PCV in the control was significantly (P<0.05) reduced in day-4 whereas in the extract treated groups significant change was not observed (P>0.05). Body weight also did not exhibit any significant(P>0.05) reduction betweeen day-0 and day-4 in the treated groups as well as in the negative control.

Table no 3: Effect of Chloroform extract of *Carissa spinarum* on PCV and body weight of *P. berghei* infected mice

Dose mg/kg extract	PCV			Body weight		
	Day-0	Day-4	Mean % change	Day-0	Day-4	Mean % change
NC	50.00±1.15	40.17±3.92*	-19.66±2.6[a]	37.1±30	35.9±0.5	-3.23±1.9[a]
200	58.05±2.46	55.71±1.43	-4.03±5.17[b]	31.43±1.78	34.76±1.57	10.59±7.6[b]
400	56.76±3.41	49.88±1.41	-12.12±1.9[ab]	29.66±0.63	31.13±0.5	4.95±4.1[ab]
800	56.29±2.83	53.93±4.46	-4.19±3.0[b]	23.26±0.48	23.97±3.33	3.05±4.9[ab]
CQ	54.73±0.27	53.37±0.21	-2.48±1.96[b]	35.8±0.23	34.97±0.92	-2.3±0.34[ab]

* =there was significant change between day-0 and day-4 (P <0.05)
Means in a column followed by the same letter do not differ significantly (P>0.05)
Key=Values are presented as M±SEM; n=5; CQ= Chloroquine Phosphate; PC= Positive control; NC=negative control (0.2ml of dH2O).

Table no 4 shows the antimalarial effect of hydro-alcholic extract(lower doses) of *C. spinarum* on Day-4 post infection and mean survival time**.** Hydro-alcoholic and chloroform extracts of the plant produced a dose dependent suppressive effect at various doses employed. The crude hydro-alcoholic (80% methanol) extract (100 mg; 200 mg; 400 mg/kg) of *C. spinarum* significantly (P<0.05) suppressed the parasitaemia but did not improve survival time significantly (P>0.05).

Table no 4: Antimalarial activities of hydroalcholic extract of *C. spinarum* on Day-4 post infection and mean survival time (lower doses)

Dose mg/kg extract	% Parasitaemia ± SEM	Mean % Suppression	MST(days)
NC	50.84±2.11[a]	0.00[a]	7.6±0.2[a]
100	34.68±1.42[b]	31.78±4.21[b]	8.8 ±.67[a]
200	32.57±2.81[bc]	37.21±3.35[bc]	9.4±0.67[a]
400	28.55±0.89[c]	42.08±2.34[c]	9.6±1.51[a]
QC(PC)	0.00[d]	100[d]	30(+)[b]

Means in a column followed by the same letter do not differ significantly (P>0.05)
Key: Values are presented as M±SEM; n=5; CQ= Chloroquine Phosphate; PC=Positive control; NC=negative control (0.2ml of dH2O); + = maximum days for follow-up; MST=Mean survival time

Table no 5 shows the Antimalarial activities of hydro-alcoholic extract (higher doses) of *C. spinarum* on Day-4 post infection and mean survival time The higher doses (600, 800 and 1000 mg/kg) of hydro-alcoholic extracts of *C. spinarum* showed significant (P<0.05) suppression and at the dose of 1000 mg/kg it exhibited highly significant suppression (P<0.001).It also significantly (P<0.05) improved the mean survival time of the treated groups.

Table no 5: Antimalarial activities of hydro-alcoholic extract of *C. spinarum* on Day-4 post infection and mean survival time (higher doses)

Dose mg/kg extract	% parasitaemia ± SEM	Mean % suppression	Mean Survival Time
NC	33.53±0.49[a]	0.00[a]	8.4±0.24[a]
600	17.41±2.03[b]	46.15±1.5[b]	11.5±1.04[b]
800	14.33±2.34[bc]	55.75±2.28[bc]	12.6±1.2[b]
1000	11.8±2.80[c]	62.88±2.34[c]	14.0±1.4[b]
QC(PC)	0.00[d]	100[d]	30(+)[c]

Means in a column followed by the same letter do not differ significantly (P>0.05)
Key: Values are presented as M±SEM; n=5; CQ= Chloroquine Phosphate; PC= Positive control NC=negative control (0.2ml of dH2O); + maximum days of follow up;

Table no 6 Antimalarial effects of chloroform extract of *C. spinarum* on against Plasmodium parasite and its impact on mean survival time. The chloroform crude extract (200, 400) of *C. spinarum* exhibited a significant (P<0.05) suppression of the parasitaemia and suppression was highly significant (P<0.01) at the dose of 800 mg/kg. Moreover, the treated mice showed significantly (P<0.05) improved mean survival time in comparison with that of the negative control.

Table no 6: Antimalarial activities of chloroform extract of *Carissa spinarum* on Day-4 post infection and mean survival time

Dose mg/kg of bwt/day	% Parasitaemia ± SEM	% Suppression± SEM	M ST
NC	42.77±1.54[a]	0.00[a]	7±0.5[a]
200	30.8±1.58[b]	28±4.29[b]	10±1.7[b]
400	27.1±1.29[b]	36.64±2.33[b]	11±0.8[bc]
800	17.84±0.85[c]	58.29±2..07[c]	12.5±0.41[c]
QC(PC)	0.00[d]	100[d]	30(+)[d]

Means in a column followed by the same letter do not differ significantly (P>0.05)
Key: Values are presented as M±SEM; n=5; CQ= Chloroquine Phosphate; PC=Positive control; NC=negative control (0.2ml of 20% DMSO); += maximum days for follow-up

IV. Discussion

The hydro-alcoholic and chloroform extracts of *C. spinarum* exhibited comparable suppressive activity on *P. berghei*. Similar result has been reported on elsewhere in which *in vitro* study of methanol crude extract of the plant was reported to be effective[21]. The highest suppression in the plant extract was shown at the maximum dose given of 1000 mg/kg. This could possibly be due to the fact that the active compounds, responsible for the antimalarial activity, mostly occur in low levels in natural products and their activity may not have significant impact in lower doses [22]. It is noteworthy to indicate that the antimalarial activities observed in the plant extract are from hydrophilic extracts because these extracts are closer in composition to the aqueous preparations commonly used by community[23].

In addition to the suppression test, different parameters are used when evaluating antimalarial activity of a given plant extract in animal model. Body weight loss is one feature of rodent malaria infections[24]. The crude chloroform and higher doses of the hydro-alcoholic extract of *C. spinarum* prevented weight loss; however, the lower doses of hydro-alcoholic extract of *C. spinarum* did not prevent weight loss. In addition to the body weight, another parameter used when evaluating antimalarial activity in animal model is packed cell volume[25]. In the present study, the higher hydro-alcoholic doses (600, 800 and 1000 mg/kg) and chloroform extracts of *C. spinarum* prevented significant PCV reduction in a dose dependent manner; nevertheless the lower doses (i.e. 100, 200 and 400 mg/kg) the hydro-alcoholic extract of *C. spinarum* did not prevent PCV fall. The effect of rodent malaria on PCV as measured by hematocrit was parasite-induced, which is occurred after infection[26].

The hydro-alcoholic and chloroform extracts of *C. spinarum* significantly prolonged the mean survival time of the study mice indicating that the extracts suppressed *P. berghei* and reduced the overall pathologic effect of the parasite on the study mice in a dose related manner[27]. This could indicate that the half-life of crude extract of *C. spinarum* is high. Plants possess different classes of secondary metabolites that are responsible for antimalarial activity, but the most important and diverse biopotency has been observed in alkaloids, quassinoids and sesquiterpene lactones [28]. The ethanolic and methanolic extracts of the root bark of *C. spinarum* was found to have alkaloids, steroids, terpens, flavoinds, tannis, and saponins [29,30] and the chloroform extract of the root bark of the plant was reported to have alkaloids, saponins and terpenoids[30] which have been implicated in antimalarial activity in other plants. Biological actions can be due to the phytochemical components in complicated concert of synergistic or antagonistic activities these secondary metabolites[31].

Results with chloroquine indicate that the malarial model used in this study is sensitive towards antimalarial agent and therefore validate its use as a standard drug in screening of antimalarial activities from plant sources [32]. In oral administration of the hydro-alcoholic extract of *C. spinarum* in the dose of 5000 mg/kg for the acute toxicity did not produce any significant physical and no death was recorded within 24 hours. This result is in agreement with the report of other studies[33, 34]. The plant can be considered as safe according to the Organization for Economic Cooperation and Development[14]. This could substantiate the traditional use of this plant in the folk medicine of Ethiopia as indicated in ethnobotany study[9].

V. Conclusion

The Hydro-alcoholic and chloroform extracts from C. *spinarum* exhibited antimalarial activity as seen in their ability to suppress *P. berghei* infection in mice in dose dependent manner. Antimalarial activities as well as the lack of acute toxicity of the extracts found in the present study may validate use of the plant in traditional medicine against malaria and suggest its ethno-pharmacological usefulness as antimalarials. The active ingredients responsible for the activity should be isolated by different extraction methods.

Acknowledgment

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