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Research

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In vivo antimalarial activity of crude fruit extract of *Capsicum frutescens* var. minima (Solanaceae) against *Plasmodium berghei* infected mice

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Abstract

Background:

The alarming spread of drug resistance to current antimalarial agents is threatening malaria controlling efforts. This, consequently, urged the scientific community to discover novel antimalarial drugs. Successful and most potent antimalarial drugs were obtained from medicinal plants. *Capsicum frutescens* is claimed to possess an antiplasmodial activity in Ethiopian and Ugandan folkloric medicine. However, there is lack of pharmacological evidence for its antiplasmodial activity. This study, hence, was aimed at evaluating the *in vivo* antiplasmodial activity of *C. frutescens* in mice model.

Methods: The 4-day suppressive test was employed to ascertain the claimed antiplasmodial effect of the plant. Following inoculation with *P. berghei*, mice in treatment groups were provided with three dose levels (100, 200 and 400 mg/kg) of the extract. Whereas, 2% Tween80 and chloroquine served as negative and positive control, respectively. Weight, temperature, packed cell volume, parasitemia and survival time were then monitored.

Results: The oral acute toxicity study revealed that the crude extract caused no mortality and revealed no overt sign of toxicity. In 4-day suppressive test, all dose levels of the extract was found to exhibit a significant ($p<0.05$) inhibition of parasitemia compared to negative control. Maximum parasite suppression (93.28%) was exerted by the highest dose (400mg/kg/day) of extract. In addition, the extract significantly ($p<0.05$) prolonged survival time and prevented body weight loss, reduction in temperature and anemia compared to vehicle treated group.

Conclusion: This investigation found a strong evidence that fruit extract of *C. frutescens* is endowed with a promising antiplasmodial activity. Hence, the plant could serve as a potential source of newer antimalarial agent.

Key words: *C. frutescens*, antimalarial, 4-day suppressive test, crude extract, *Plasmodium berghei*

Background

Malaria still continued to pose public health risks throughout the globe. This disease is estimated to infect half of the world population. The burden is heaviest in sub-Saharan Africa. It is still the leading cause of outpatient visits and hospital admission in this region. Moreover, an estimated 90% of malaria deaths occur in this area [1, 2]. Similarly, there is a high risk of malaria in Ethiopia. Only 25% of Ethiopian population live in areas that are free of malaria [3-5].

The burden from the disease is exaggerated among children under the age of five and pregnant women than adults [6-9]. Besides to its public health burden, the disease has also negative socioeconomic consequences [1, 2, 4]. The economic impact includes costs of health care, days lost in education, decreased productivity, and loss of investment, tourism and trade. During the last decade alone, over 19 billion US dollar was invested by governments of malaria endemic countries and international partners to tackle the disease [2-4].

Emergence of resistant malaria parasites to the conventional antimalarial drugs become the major challenges in controlling this disease. Previously sensitive parasites are developing resistant to several antimalarial drugs [10]. More importantly, there are alarming reports on parasite resistance to currently existing first line drug regimen, ACTs, in parts of Cambodia and Thailand as well as in certain parts of Africa [11, 12]. Furthermore, these resistant strains have the potential to spread to the rest of the world. This, therefore, necessarily triggers a search for new and effective antimalarial drugs [11-13].

Historically, successful and most potent antimalarial drugs were developed from medicinal plants, which led to study of plants as main source of new antimalarial drugs [14-16]. In malaria-endemic countries, natural and traditional medicines, mainly consisting of plants, are commonly used to treat malaria. More than 160 plant families are documented as traditional medicines used for malaria treatment [17]. Several African plants are scientifically proven to possess antimalarial properties [18].

Capsicum frutescens var. minima (Figure 1) has a claimed folkloric medicinal value in the management of malaria and other diseases in Africa [19, 20]. Nonetheless, there is lack of scientific evidences that verified the claimed antiplasmodial activity of this plant in experimental animals. Thus, this study investigated the *in vivo* antiplasmodial activity of *C. frutescens*, the

variant commonly used in Sasiga District of Ethiopia as an antimalarial remedy, against chloroquine sensitive *Plasmodium berghei* (ANKA) infection in mice.

Methods and materials

Experimental plant collection

For authentication purpose, the branch of *C. frutescens* with its leaves and fruits attached were, first, collected from Sasiga District, western Ethiopia on January, 2018. After authentication, the fresh fruits of the plant were collected for extraction. The fruits were carried in a plastic bag during transportation. Identification and authentication of the plant was done by a taxonomist at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University, where a voucher specimen was coded (GH 02/2018) and deposited for future reference.

Preparation of the crude extract

The fresh fruits of the plant were first thoroughly rinsed with tap water and cleaned with gauze to remove debris. The fruits were then air dried under shade at room temperature and pulverized using mortar and pestle to make a coarse powder for extraction. Next, the 80% methanolic crude extract was prepared using cold maceration technique as per to the method described by Bello et al. (19). Accordingly, 500g of coarse powder was soaked in 750 ml of 80% methanol solution using an Erlenmeyer flask. The extraction process was facilitated using a mechanical shaker (Bibby Scientific Limited Stone Staffo Reshire, UK) at 120 rpm for 72 hours.

After 72 hours, the resulting distillate containing the 80% methanol crude extract was separated from the marc with gauze, and further filtered by Whatman filter paper number 1 (Whatman®, England) under suction filtration. This procedure, in the same solvent system, was repeated twice using the marc left. The filtrate was then transferred to a round bottom flask and concentrated using a rotary evaporator (Buchi Rota vapor R-200, Switzerland) under reduced pressure. Then, the resulting extract was frozen in deep freezer overnight and freeze dried with a lyophilizer (Operan, Korea Vacuum Limited, Korea) to remove water at -50°C and vacuum pressure of 200 mBar. The concentrated extract was transferred into an amber glass bottle and kept at -20°C until use. The percentage yield was calculated using the following formula:

$$Pecentage\ yield = \frac{weight\ of\ dried\ extract}{weight\ of\ pulverized\ fruit\ used\ in\ maceration} * 100$$

Animals in the experiment

The experiment was conducted on Swiss albino mice whose weights were in a range between 25 and 31g, and 6 to 8 weeks old. The animals were those obtained from the Ethiopian Public Health Institute. Before conducting the experiment, acclimatization of the animals for a week to the experimental condition was done. The experiment was conducted in animal house at Addis Ababa University, College of Health Sciences, School of Pharmacy. Polypropylene cages were utilized to house the animals where they stayed at 12 hours light-dark cycle. A standard pellet food was given regularly to the mice while water was provided *ad libtum*. The standard animal handling practice utilized in the present study was following the well accepted animal handling protocol [21].

Test for acute toxicity

To determine the safety of the plant extract, the Organization for Economic Cooperation and Development number 425 guideline [22] was used to evaluate the oral acute toxicity profile. In this test, after weighing a single overnight fasted mouse, 2000mg/kg of the crude extract was provided to the mouse using oral gavage at once. Then after, food was withheld for 2 hours but not water. The mouse was, then, observed continuously for the first 30 min and intermittently for 4 h, over a period of 24 h. As neither death nor any signs of acute toxicity was seen, similar dosage of the extract was provided to another four female mice. Then, the mice were followed for 2 weeks to see if changes that are signs of acute toxicity occur.

***In vivo* antimalarial screening**

Grouping and dosing of animals

For evaluating the *in vivo* antiplasmodial effect of the 80% methanol crude extract of the fruit of *C. frutescens*, Swiss albino mice were used. Thirty male mice were intraperitoneally infected with *P. berghei* and randomly divided in to five groups of six mice each, in separate cages. Negative control mice received 10ml/kg dose of solvent for reconstitution (Tween 80 2% v/v). Mice in treatment groups were provided with three dose levels (100, 200 and 400 mg/kg) of the extract. Mice in the positive control were treated with 10mg/kg dose of chloroquine (CQ) base. Dose selection for the extract was determined based on the result of acute toxicity and preliminary screening test done on the plant extract.

Inoculation of the parasite

P. berghei (ANKA strain) sensitive to chloroquine was obtained from Ethiopian Public Health Institute. Then, until 30-37% of parasitemia level was attained, the parasites were maintained by serial passage of blood from infected mice to non-infected ones every week [23]. The parasitaemia level of the donor mice were determined from the blood collected by cutting a 0.5 to 1mm section from tail of the mice with scissors. Mice with the level of parasitemia mentioned above were used as a donor. Donor mice was euthanized using halothane in a closed chamber and infected blood was immediately collected through cardiac puncture into heparinized vacutainer tube [24- 26]. The collected blood from all donor mice were pooled together to avoid variability and then diluted in normal saline [25]. The blood was then diluted with physiological saline (0.9%) in such a way that the final suspension would contain about 1×10^7 parasitized red blood cells (PRBCs) in every 0.2 ml of blood [27]. Experimental animals were inoculated with 0.2 ml of diluted blood, intraperitoneally.

The 4-day suppressive test

In vivo schizontocidal activity of the extract against early chloroquine sensitive *P. berghei* infection was carried out according to the method described by Peter et al. [24]. On the first day (D_0), apparently healthy mouse in each group was coded and inoculated with 0.2 ml of blood containing about $1x10^7$ *P. berghei* infected RBCs. Then, three hours after infection (D_0), each animal in each group was weighed and orally provided with the corresponding dosage of the extract and the controls using oral gavage for four consecutive days (D_0-D_3). At the beginning of D_0 and/or at the end of the 4^{th} day (D_4), parameters detailed below were determined and the mice were monitored daily for 30 days to monitor their survival time [24].

Parasitemia and survival time determination

At D_4, blood was collected from the tail of each mouse using clean and non-greasy slides to prepare thin blood films. After allowing the slides to air-dry, slides were viewed microscopically using the X100 objective. The percentage parasitemia (PP) was obtained by counting the number of PRBCs out of erythrocytes in random fields of the microscope. Two stained slides for each mouse were examined. Three fields with approximately 200-500 cells were counted for each slide and PP for each mouse was determined using the following formula [28, 29]:

$$PP = \frac{PRBC}{total\ number\ of\ RBCs\ counted} \times 100$$

The mean percentage parasitemia suppression (PPS) was calculated using the formula described below [27, 29]:

$$PPS = \frac{(\text{mean } PP\ in\ negative\ control - \text{mean } PP\ in\ treatment\ group)}{mean\ PP\ in\ negative\ control} \times 100$$

The mean survival time (MST) for each group was calculated as follows [16]:

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

Weight, temperature and packed cell volume determination

Weight and rectal temperature of each mouse were recorded just before treatment, and after treatment on D_4. The mean percentage changes were then calculated and analyzed for each group [16, 25]. In the same way, packed cell volume (PCV) was measured before inoculation and after treatment. To determine PCV, blood was collected from the tail of each mouse in heparinized microhaematocrit capillary tubes. The capillary tubes were filled to $3/4^{th}$ of their height with blood and sealed with sealing clay at their dry end. The tubes were then placed on a micro-hematocrit centrifuge (Centurion Scientific, UK) with the sealed end facing the periphery and centrifuged at 11,000 rpm for 5 minutes [26]. Finally, PCV was determined using the standard hematocrit reader (Hawksley and Sons, England) according to the formula indicated below [28, 29]:

$$PCV = \frac{volume\ of\ erythrocyte\ in\ a\ given\ volume\ of\ blood}{total\ blood\ volume\ examined}\ X\ 100$$

Phytochemical screening

The phytochemical constituents of the 80% methanolic crude fruit extract of *C. frutescens* were investigated qualitatively and quantitatively following standard methods [30-32]. The major plant phytochemicals including alkaloids, saponins, tannins, flavonoids, glycosides, steroids and terpenoids were assayed.

Analysis of data

After organizing, data were fed in to SPSS version 22 and then analyzed. To compare the mean PPS, MST, changes in mean body weight, PCV and rectal temperature of the *P. berghei* infected mice between the extract received groups and the controls, and among extract received groups, one way analysis of variance (ANOVA) followed by *Tukey post Hoc* test was done. P-value less than 0.05 was considered to be statistically significant with analysis at 95% confidence interval.

Results

Percentage yield of the extract

The physical nature of the extract was found to be dry brownish powder. A total of 97.9g (19.58 % yield) of the extract was harvested from the 80% methanolic crude extract of the fruits of *C. frutescens*.

Test for acute toxicity

Test for acute toxicity showed that no mortality was observed within the first day and the next 2 weeks period of observation. Furthermore, the plant caused no visible signs of acute toxicity as evidenced by the gross behavioral and physical observation of the experimental mice.

The 4-day suppressive test

Parasitemia and survival time determination

The effect on survival time and parasite load by the plant extract is summarized below (Table 1). All dose levels of the crude fruit extract evaluated in the study exhibited a statistically significant ($p<0.05$) difference in reducing parasite load compared to negative control, in a dose-dependent fashion. The 400 mg/kg/day dose of the extract exhibited the highest parasitemia inhibition

(93.28%) compared to other doses. Nevertheless, the effect produced by the crude extract was inferior to the standard drug, which cleared the parasite to undetectable level. In addition, the longest mean survival time (27.42 days) was exhibited at the highest dose administered. CQ treated groups, on the other hand, survived throughout the monitoring period (>30days).

Weight, temperature and packed cell volume determination

All the three doses of the extract significantly ($p<0.05$) averted body weight loss compared to the negative control (Table 2). Moreover, all dose levels of the extract were able to significantly prevent body temperature dropping due to parasite infection compared to those in the vehicle treated groups (figure 2). This protection against body weight loss and rectal temperature dropping was dose-dependent. Accordingly, the highest protection in both weight and temperature reduction was exhibited by the highest dose given, 400 mg/kg/day.

A dose-dependent and statistically significant ($p<0.05$) protection against reduction of PCV was exhibited by 80% methanol crude extract compared to the negative control. The highest protection against reduction of PCV was exhibited by the upper dose (400 mg/kg/day) of extract (Figure 3). The plant extract ameliorated infection induced reduction in PCV in the following order: 10 mg/kg dose of CQ> 400 mg/kg of extract> 200 mg/kg of extract > 100 mg/kg of extract.

Phytochemical screening

Preliminary qualitative phytochemical test

Preliminary phytochemical screening test done on the 80% methanolic crude fruit extract of *C. frutescens* revealed that the plant contains alkaloids, saponins, tannins, flavonoids, glycosides and terpenoids. However, steroids were absent (Table 3).

Quantitative phytochemical investigation

The study to determine the amount of each phytochemical in the 80% methanolic fruits crude extract of *C. frutescens* depicted that the plant contains the highest value of alkaloids followed by saponins (figure 4).

Discussion

More than 80 percent of African population rely on traditional herbal medicine to meet their primary healthcare demand. Nonetheless, pharmacologic investigations to standardize and set the antimalarial efficacy and safety of such plants were not largely done to this indigenous gift [3, 5]. *C. frutescens* is commonly used as a folk medicine for malaria treatment. Consequently, this study investigated the *in vivo* antimalarial activity of the plant in an attempt to contribute to the discovery of novel antimalarial drugs using 4-day suppressive test [19, 20]. The test is a widely utilized standard *in vivo* antimalarial screening model in scientific research. This is mainly because *in vivo* study takes in to account the possible pro-drug effect and involvement of immune system in killing the parasites towards the efficacy and safety of the investigational plants [28].

In the present study, crude fruit extract of *C. frutescens* showed a dose-dependent significant inhibition of parasitemia compared to vehicle treated group. This is comparable with similar studies done on *Croton macrostachyus* [26] and *Withania somnifera* [33]. However, chemoprevention efficacy of the plant is superior to our previous report on *Schinus molle* [16].

Phytoconstituents such as alkaloids, abundantly localized in the plant extract, could be responsible for the antiplamodial activity as plant derived alkaloids such as quinine are evidenced to possess a potent antimalarial activity. This plant also contain terpenoids, phenolic compounds and flavonoids. These bioactive principle have been reported to possess a range of antimalarial activity in the literature [34-38].

In discovering antimalarials from plants, the extract is ideally expected to prevent reduction in PCV, body weight and body temperature due to the development of parasitemia [27]. Though a significant prevention of body weight loss was exhibited by crude extract, the mean value of the body weight showed reduction in treatment groups on D_4 as compared to D_0. This could be ascribed to the inability of the extract to completely eradicate the pasrasite load [16, 30]. PCV was monitored to assess the ability of the plant in ablating malaria induced hemolysis. The lowest dose exhibited the least protection against hemolysis compared to the other two upper doses. This could be perhaps because of the abandunt phytoconstituents concentrated in the upper doses.

The three doses of methanolic crude extract significantly prevented rectal temperature dropping

due to parasitemia as compared to the vehicle. These activities probably indicate that the extract ameliorate some pathological processes that cause reduction in internal body temperature and metabolic rates. In 4-day suppressive test, a candidate antimalarial agent should prolong survival time to be active antimalarial agent. The extract significantly prolonged the survival time of mice as compared to the control. However, unlike CQ, the mice were not cured. This might be due to the incomplete clearance of parasite or short half-life profile of active constituents [16, 26, 29].

A candidate antimalarial agent should elicit parasite suppression of 30% or greater [39]. Hence, the extract at all doses tested was found to possess a promising chemosuppression effect and found to be active in counteracting the malaria parasite. Furthermore, if the antimalarial activity of a compound displayed a percent growth inhibition of ≥50% at a dose of 500-250, 250-100 and ≤100 mg/kg/day, literature grades it as moderate, good and very good, respectively [16, 39, 40]. Therefore, the fruit of *C. frutescens* possesses a very good antimalarial activity.

It can be deduced from the acute toxicity test that the oral medial lethal dose (LD_{50}) of the extract could be greater than 2000 mg/kg of the extract as per OECD guideline No 425 [22]. Evidence from this data could serve as a justification for the safe folkloric use of the fruit of *C. frutescens* for the treatment of malaria by the local people in Ethiopia and Uganda [20, 22].

Conclusion

The findings of the present study indicated that the fruits of *C. frutescens* has a promising *in vivo* antimalarial activity that can serve as a potential source to develop effective and safer antimalarial drugs. The highest chemosuppressive effect was exhibited by the upper dose of the tested extract indicating the presence of high concentration of bioactive principles in this dose. Moreover, the data would provide evidence to indorse the traditional use of the plant by the local communities for the treatment of malaria in Ethiopia and Uganda.

Abbreviations

ANOVA: analysis of variance

MST: mean survival time

PCV: packed cell volume

PP: percentage parasitemia

PPS: percentage parasitemia suppression

PRBCs: parasitized red blood cells

SEM: standard error of the mean

SPSS: statistical package for social sciences

WHO: World Health Organization

Authors' contributions

GH performed the experiment, analyzed and interpreted the data. GH prepared the draft and SA reviewed it. All authors participated in the write up of the manuscript. All authors have read and approved the final manuscript.

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Ethics approval and consent to participate

Ethical clearance was granted by the Ethical Review Committee of the School of Pharmacy, College of Health Sciences, Addis Ababa University.

Consent for publication

Not Applicable

Availability of data and materials

The data sets supporting the conclusion of this study are available from the corresponding author on reasonable request.

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Competing interests

The authors of this work declare that they have no competing interests.

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Figures 1 The picture of the fruits of *Capsicum frutescens* var. minima

Figures 2 Changes in body temperature of mice infected with *P. berghei* and treated with the 80% methanolic crude fruits extract of *Capsicum frutescens*

Data are expressed as mean ± SEM (n=6); a, compared to TW 10 ml/kg; b, compared to 100 mg/kg; c, compared to 200 mg/kg; d, compared to 400 mg/kg; e, compared to CQ10; *p<0.05. TW, 2% tween 80; CQ, chloroquine base; 80ME, 80% methanol extract; D0, pre-treatment value on day 0; D4, post-treatment value on day four. The numbers in rectangles across the graphs show the change in mean temperature between D0 and D4.

Figure 3 Changes in packed cell volume of mice infected with *P. berghei* and treated with the 80% methanolic crude fruits extract of *Capsicum frutescens*

Data are expressed as mean ± SEM (n=6); a, compared to TW 10 ml/kg; b, compared to 100 mg/kg; c, compared to 200 mg/kg; d, compared to 400 mg/kg; e, compared to CQ10; *p<0.05. TW, 2% tween 80; CQ, chloroquine base; 80ME, 80% methanol extract; PCV, packed cell volume; D0, pre-treatment value on day 0; D4, post-treatment value on day four. The numbers in rectangles across the graphs show the change in mean PCV between D0 and D4.

Figure 4 Quantitative determination of phytochemicals in the 80% methanolic crude fruits extract of *Capsicum frutescens*

Alk, alkaloids; Tan, tannins; Sap, saponins; Flav, flavonoids; Terp, terpenoids; Gly, glycosides.

Table 1 The 80% methanolic crude fruit extract of *Capsicum frutescens* consequences on parasite load and the length of survival of mice infected with *P. berghei*

Group	% Parasitemia	%Suppression	Survival Time (day)
CON	57.84±1.48	0.00	6.25±0.55
80ME100	15.82±0.76	72.65^{a*,c*,d*,e*}	17.66±0.37^{a*,d*,e*}
80ME200	7.01±0.91	87.88^{a*,b*d*,e*}	22.02±0.51$^{a*, d*,e*}$
80ME400	3.88±0.85	93.29^{a*,b*,c*,e*}	27.42 ±0.74^{a*,b*,c*,e*}
CQ10	0.00±0.00	100.00 ^{a*}	>30.00±0.00 ^{a*}

Data are expressed as mean ± SEM (n = 6); a, compared to CON; b, compared to 100 mg/kg; c, compared to 200 mg/kg; d, compared to 400 mg/kg; e, compared to CQ10; $^{*}p<0.05$. 80ME = 80% methanol crude extract, CON= negative control, CQ=chloroquine base. Numbers next to letters (10,100,200,400) in the first column refer to dose in mg/kg.

Table 2 Changes in weight of mice infected with *P. berghei* and treated with the 80% methanolic crude fruits extract of *Capsicum frutescens*

Group	Weight (g)		
	D_0	D_4	Change
CON	28.84±0.65	25.24±0.45	-3.60±2.02
80ME100	29.12±0.82	28.86±0.42	-0.26±0.14^{a*c*d*e*}
80ME200	28.92±0.71	28.82±0.31	-0.10±0.20^{a*b*d*e*}
80ME400	29.21±0.49	29.19±0.23	-0.02±0.15^{a*b*c*e*}
CQ10	29.03±0.80	29.33±0.56	0.30±0.05^{a*}

Data are expressed as mean ± SEM (n = 6); a, compared to CON; b, compared to 100 mg/kg; c, compared to 200 mg/kg; d, compared to 400 mg/kg; e, compared to CQ10; $^{*}p<0.05$. D_0 = pre-treatment value on day 0, D_4 = post-treatment value on day four. 80ME = 80% methanol crude extract, CON=negative control, CQ=chloroquine base. Numbers next to letters (10,100,200,400) in the first column refer to dose in mg/kg.

Table 3 Preliminary qualitative phytochemical screening of the 80% methanolic fruits crude extract of *Capsicum frutescens*

Phytoconstituents	Test result
Terpenoids	+
Steroids	-
Flavonoids	+
Saponins	+
Glycosides	+
Tannins	+

Alkaloids	+

-, shows absence of phytoconstituents; +, shows presence of phytoconstituents

Figures

Figure 1

The picture of the fruits of Capsicum frutescens var. minima

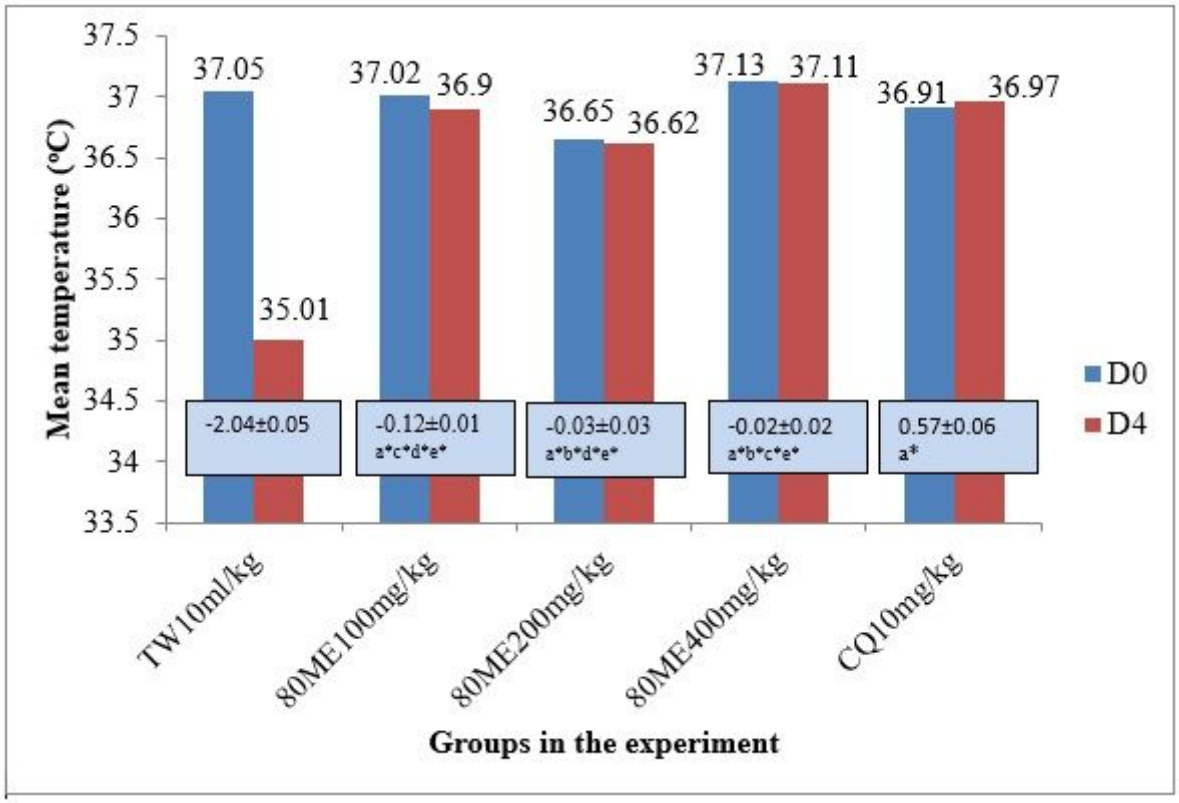

Figure 2

Changes in body temperature of mice infected with P. berghei and treated with the 80% methanolic crude fruits extract of Capsicum frutescens Data are expressed as mean ± SEM (n=6); a, compared to TW 10

ml/kg; b, compared to 100 mg/kg; c, compared to 200 mg/kg; d, compared to 400 mg/kg; e, compared to CQ10; *p<0.05. TW, 2% tween 80; CQ, chloroquine base; 80ME, 80% methanol extract; D0, pre-treatment value on day 0; D4, post-treatment value on day four. The numbers in rectangles across the graphs show the change in mean temperature between D0 and D4.

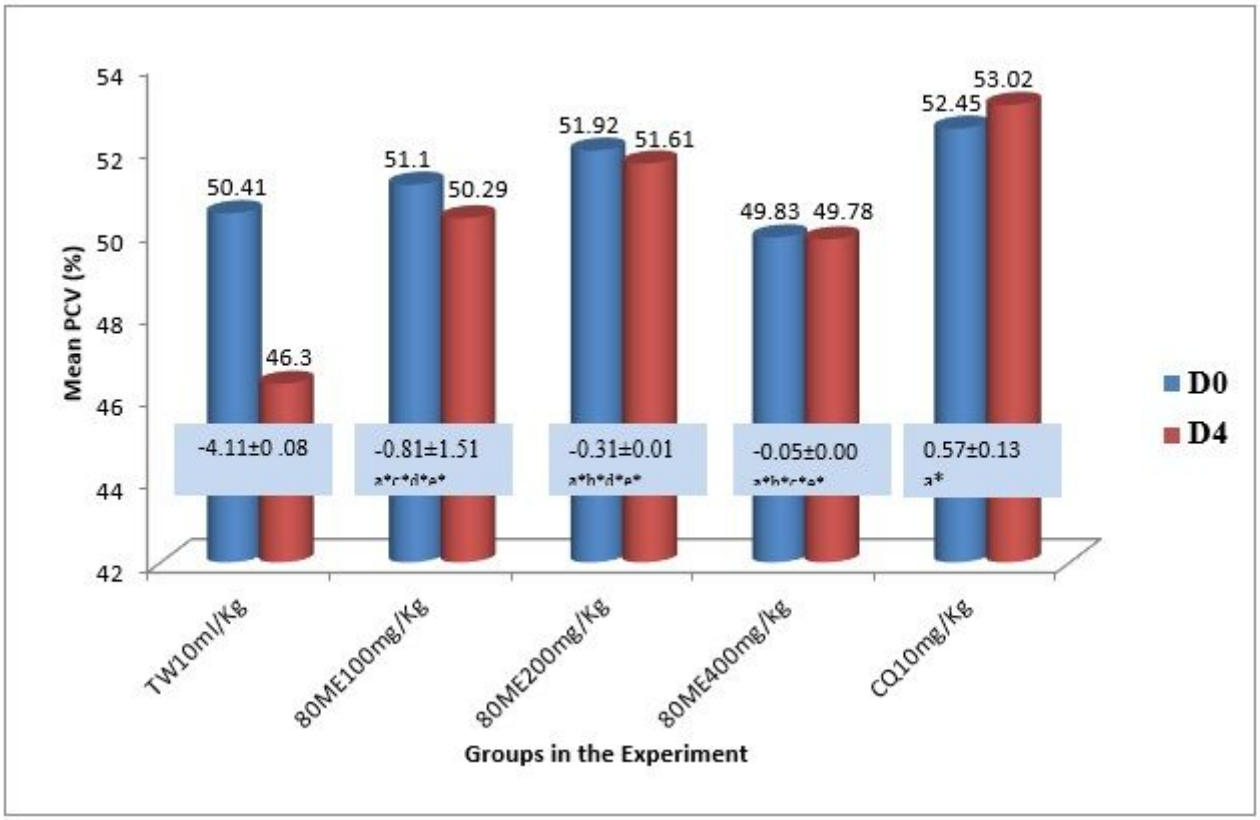

Figure 3

Changes in packed cell volume of mice infected with P. berghei and treated with the 80% methanolic crude fruits extract of Capsicum frutescens Data are expressed as mean ± SEM (n=6); a, compared to TW 10 ml/kg; b, compared to 100 mg/kg; c, compared to 200 mg/kg; d, compared to 400 mg/kg; e, compared to CQ10; *p<0.05. TW, 2% tween 80; CQ, chloroquine base; 80ME, 80% methanol extract; PCV, packed cell volume; D0, pre-treatment value on day 0; D4, post-treatment value on day four. The numbers in rectangles across the graphs show the change in mean PCV between D0 and D4.

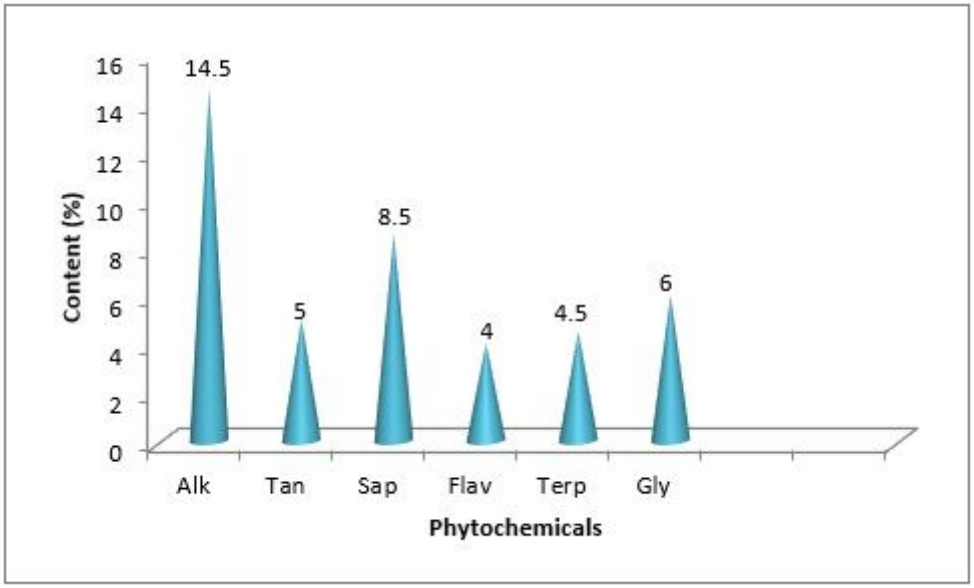

Figure 4

Quantitative determination of phytochemicals in the 80% methanolic crude fruits extract of Capsicum frutescens Alk, alkaloids; Tan, tannins; Sap, saponins; Flav, flavonoids; Terp, terpenoids; Gly, glycosides.