

Full Length Research Paper

Antimalarial effects of *Diospyros chamaethamnus* and *Guibourtia coleosperma* extracts in mice with *Plasmodium berghei* malaria

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The development of effective antimalarials remains crucial due to ineffective treatments, increasing drug resistance, and restricted access to these drugs in resource-poor malaria-endemic countries. This study aimed to investigate the efficacy of *Diospyros chamaethamnus* and *Guibourtia coleosperma* based on their traditional uses. Efficacy of the extracts was evaluated against *Plasmodium berghei* in Swiss albino mice using suppressive and prophylactic tests. The plant extracts were also tested for toxicity in healthy mice, beginning with a dose of 300 mg/kg. The plant extracts demonstrated antiplasmodial activity at an 800 mg/kg dose, with *D. chamaethamnus* showing 44.66% and *G. coleosperma* 29.59%. This dose prolonged the survival of the mice post-infection by 50 and 58%, respectively, compared to control untreated mice ($P < 0.05$). Aqueous extracts (800 mg kg⁻¹) of *D. chamaethamnus* and *G. coleosperma* showed prophylactic activities, reducing parasite load by 56.13 and 55.48%, respectively. No mortalities or adverse effects were observed in the mice, indicating that the extracts were not toxic. According to the findings, the two plants show promise as alternative malaria treatment options.

Key words: Malaria, antiplasmodial activity, suppressive test, repository test, acute oral toxicity, *Diospyros chamaethamnus*, *Guibourtia coleosperma*, Namibia.

INTRODUCTION

Malaria is an infectious disease caused by *Plasmodium* parasites. It accounts for about 249 million cases and 608,000 deaths worldwide, despite being preventable and treatable (World Health Organization 2023). Most cases (93.6%) and deaths (95.4%) occur in Africa

(World Health Organization, 2023) significantly impacting the economies of developing countries by depleting healthcare resources and reducing economic activity (Arrow et al., 2004; Onwujekwe et al., 2013; Kayiba et al., 2021). In Namibia, malaria is prevalent in the north-

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central and northeastern regions, which are home to over half the population. The climate in these areas supports the breeding of Anopheline mosquitoes and the transmission of *Plasmodium falciparum* Welch, 1897 (Ministry of Health and Social Services, 2017). Despite the health threat posed by malaria, Namibia is making progress in its efforts to eliminate the disease (Smith et al. 2021). The success of this effort can largely be attributed to the implementation of various malaria control and prevention strategies, including surveillance, transmission-blocking measures, and treatment programs (Shretta et al., 2017). Treating all malaria cases with effective medicines is crucial for eliminating *Plasmodium* reservoirs. This treatment is a key tool for eradicating the disease (Shretta et al., 2017). However, resistance or partial resistance to artemisinin derivatives in artemisinin-based combination treatments has been reported (World Health Organization 2023) and would reverse the global efforts in malaria control, as well as negatively affect the health of communities in malarious areas (Shretta et al., 2017; Ouji et al., 2018; Dhiman, 2019).

Furthermore, the World Health Organization (WHO) recommends treatment for malaria within 24 h of fever onset or diagnosis (Simba et al., 2018). As a poverty-related disease, obtaining medication can be challenging (Chuma et al., 2010). Patients often travel long distances to reach a clinic, and public healthcare facilities often run out of essential drugs (Chuma et al., 2010), leading to treatment delays and potentially serious consequences. Traditionally, malaria has been treated using plant-based medicines since ancient times (Orok et al., 2021). This is exemplified by the current WHO-recommended treatment, artemisinin-based combination therapies (ACTs), which include a natural compound, artemisinin, paired with a synthetic partner drug, while the previous first-line treatment, chloroquine, is a synthetic derivative of a plant-based compound. Some communities today prefer traditional medicine over allopathic care (Chuma et al., 2010; Graz et al., 2011). Some combine the two (Traore et al., 2021). In some cases, communities may not have easy access to modern medicine. Incorporating medicinal plant use in healthcare programs could address some of these challenges, but it may be difficult to implement as there is a lack of scientific evidence of their therapeutic applications. Despite this, plant-based medicines remain a significant source of antimalarials.

Diospyros chamaethamnus Dinter ex Mildbr. and *Guibourtia coleosperma* (Benth.) J. Leonard are used to treat malaria and its clinical symptoms in malaria-endemic regions of Namibia. *D. chamaethamnus* known as Mundjongolo (Silozi) is used in combination with three other plants to treat or alleviate headaches (Table 1). Furthermore, its roots and leaves are used alongside *Ochna pulchra* Hook., *Burkea africana* Hook., and *Combretum zeyheri* Sond. for treating malaria. *G. coleosperma* referred to as Muzauli (Silozi), on the other

hand, is used to treat shivering and sweating (Table 1). In the Ohangwena region of Namibia, the leaves, bark, and roots are used to treat headaches, fever, coughs, and malaria (Nafuka and Mumbengegwi, 2013). The leaves are also used in managing severe coughs (Quattrocchi, 2016), and the leaves and roots together with those of *Securidaca longipedunculata* Fresen. and *Pterocarpus angolensis* DC. for fever and malaria. This study was therefore conducted to provide scientific evidence of the traditional uses of these plants. It aimed to assess the efficacy of *D. chamaethamnus* and *G. coleosperma* using a small animal model for malaria and acute oral toxicity in healthy mice. *Plasmodium berghei*-infected Swiss albino mice weighing 20±4 g were treated with the plant extracts pre- and post-infection. The plant extracts were evaluated for their toxicity using dose-escalation.

MATERIALS AND METHODS

Selection and collection of plants

Fresh roots of *D. chamaethamnus* Dinter ex Mildbr. and *G. coleosperma* (Benth.) J. Léonard were collected in the Zambezi region of Namibia. They were selected based on their local use, which is to treat malaria and symptoms of the disease (Table 1). The identities of the plants were confirmed by botanists from the National Herbarium at the National Botanical Research Institute (NBRI) of Namibia.

Preparation of plant

The roots were dried at ambient temperature for 4-6 weeks to avoid changes in the chemical composition and properties of the plant material. The dried plant material was ground to obtain a fine powder (particle size less than 1 mm) using a heavy-duty blender and was then stored in airtight 50 mL Falcon tubes at -20°C.

Method of extraction

Organic extracts were prepared by maceration (Njateng et al., 2013). Powdered plant material was immersed in a methanol-dichloromethane (1:1 v/v) solution at a sample-to-solvent ratio of 1:20 (w/v) for 48 h at ambient temperature with occasional shaking. The plant matter was removed by gravity filtration using Whatmann qualitative filter paper, Grade 1 (110 mm). Aqueous extracts were prepared using a method of hot water extraction similar to those traditionally used. Powdered plant material was placed in distilled water (1:20 w/v), which was heated in a water bath (60°C) for 2 h. Thereafter, the plant suspension was placed in a centrifuge for 30 min at 800xg in an Avanti™ J-25 centrifuge (Beckman Coulter, USA), and filtered using gravity filtration. The plant extracts (filtrates) were placed on a rotary evaporator (Heidolph GI, Germany) at 60°C to remove the solvent, followed by freeze-drying to obtain a solid extract. The dry extracts were stored at -20°C for further analysis.

Animals and ethical approval

Swiss albino mice [*Mus musculus* L. (Muridae)] (18-24 g) were obtained from the animal house situated at the Kenya Medical

Table 1. Ethnobotanical information on medicinal plants *D. chamaethamnus* and *G. coleosperma*.

Scientific name	Voucher specimen no.	Local name*	Use in combination with*	Ethnomedicinal use	Application
<i>G. coleosperma</i>	CID11	Muzauli	Mukwenkwebo, Mungongolo	Shivering and sweating (fever and chills) in children	Oral, topical
<i>D. chamaethamnus</i>	CID12	Mundjongolo	Situnduwanga, Kabubo, Mutjokela, Mushakashela, Mufula	Headaches	Oral, steam bath, topical

*Vernacular names of plants in Silozi.

Research Institute (KEMRI), Nairobi. This research was conducted with the approval of KEMRI's Animal Care and Use Committee. The mice were fed a grower's pellet feed and had access to water provided ad libitum. The cages were lined with wood shavings, which served as bedding for the mice. The shavings were replaced twice a week or as needed to prevent the buildup of ammonia. The rodent parasite used in this study was chloroquine-sensitive *P. berghei* ANKA, obtained from the American Type Culture Collection (ATCC), USA (BEI resources).

Revival and maintenance of parasites

Healthy mice were given injections of *P. berghei* parasites after they had thawed at room temperature. Every four days, the parasites were continuously reinfested in healthy mice (a process known as serial passaging). By means of heart puncture, blood samples were obtained from infected mice exhibiting a parasitemia of 20–30%. Phosphate saline glucose (PSG) buffer was used to dilute the blood to 1% parasitaemia. Using a 21G needle, 0.2 mL of 1×10^7 (that is, 1% parasitaemia) *P. berghei*-infected red blood cells (RBCs) were injected intraperitoneally (IP) into each animal. Using thin smears created by bleeding the tail vein, the mice were observed every day for seven days in order to assess the parasitaemia. For experimental purposes, mice with parasitemia levels between 18 and 25% were employed to infect several mouse groups.

In vivo-suppressive test

For antimalarial evaluation of the plant extracts, Peter's 4-

day suppressive test (Christian et al., 2014) was employed. A total of 80 mice were employed, 40 of which were for *D. chamaethamnus* and 40 for *G. coleosperma*. On day zero (D0), each mouse received an IP inoculation with 0.2 mL of 1×10^7 *P. berghei*-infected red blood cells. The forty mice were randomly divided into eight groups, each consisting of five mice. These groups included a control group, a standard drug group, and several treatment groups. A random number generator was used to ensure an unbiased allocation process. Each group was then assigned a code to conceal its allocation during the evaluation of treatment outcomes. Different dosages of 200, 400, and 800 mg/kg/day were calculated based on the average weight of the mice in each group. The aqueous and organic extracts were resuspended in distilled water and 10% Tween 80, respectively. The doses chosen for antimalarial evaluation were selected based on protocols established in a previous study (Omagha et al. 2022), enabling the assessment of dose-dependent responses and therapeutic potential. The aqueous and organic extracts were administered orally (PO) in various doses to groups 1-6. Group 8 was given a 10% Tween 80 solution OP (vehicle) as the control, whereas Group 7 was given 25 mg/kg of chloroquine diphosphate as the positive control. Each therapy began 3 h after infection and was administered as a single dosage every day for four days consecutively (D0 to D3). Thin blood smears were prepared on day 4 (D4) to calculate the percentage of parasitaemia and growth suppression for each dosage using the formula $((A-B)/B) \times 100$, where A represents the average parasitaemia in the negative control group and B represents the average percentage parasitaemia in the test group. The median survival time (MST) for each group over a 29-day period was calculated using a Kaplan-Meier plot

(GraphPad Prism 6) and changes in the mice's weight were also recorded.

In vivo repository (prophylactic) test

The repository test was used to determine the prophylactic activity of selected plant extracts (Arrey Tarkang et al., 2014). Eight groups of five mice each were randomly selected from a total of forty animals. Before infection, each group received different treatments for three consecutive days (from D0 to D2). Groups 1, 2, and 3 received different doses of aqueous extract from *D. chamaethamnus* (200, 400, and 800 mg/kg), while Groups 4, 5, and 6 received the same doses of aqueous extract from *G. coleosperma*. Group 7 received 10 mg/kg/day of distilled water orally (as a control), while Group 8 received 10 mg/kg per day of pyrimethamine as the positive control. On day 4 (D₃), each mouse was subjected to an IP injection of 0.2 mL of 1×10^7 *P. berghei*-infected RBCs. To assess the percentage parasitaemia, thin blood smears were prepared 72 h after infection. Using GraphPad Prism 6, the median survival time (MST) for each group during a 29-day period was determined, and the weight variations in the mice were also noted.

Acute oral toxicity

Using the Acute Toxic Class Method, the plant extracts' toxicity was assessed in female Swiss albino mice that were healthy and weighed between 18 and 20 g. The initial dosage was 300 mg/kg⁻¹ (OECD, 2002). Female mice were used for the study because they are more susceptible to

chemicals than male mice (Ikuno et al., 2008). The mice were not given any food, only water, for 3 h before and 1 h after administration of the aqueous and organic plant extracts (0.2 mL) PO in a single dose. Since the plant extracts' toxicity was unknown, the extracts were given gradually, beginning with a dosage of 300 mgkg⁻¹. The mice were monitored for symptoms of toxicity, including a rough coat (hair erection), vomiting, diarrhea, foaming in the mouth, unprovoked behavior (fighting or biting), alertness, restlessness and clonic convulsions (jerking), lethargy or recumbency, and coma after treatment for the first 4 h, which are the most crucial, then over the next 24 h, and then every day for 14 days. When no deaths were observed in the mice, a higher dose was administered. Conversely, if two or more mice died at a specific dose, the LD₅₀ was estimated by using a predetermined LD₅₀ cutoffs specified in the guidelines mentioned earlier. The weights of the mice were recorded shortly before the treatments were administered and again at the end of the observation period. The critically ill mice were either sacrificed or euthanized to prevent them from suffering; they were injected intraperitoneally with pentobarbital, and killed by cervical dislocation. These mice were officially recorded as having died. The mice that survived until the end of the observation period (day 14) were also killed humanely. Post-mortem examinations were conducted on all the animals to macroscopically assess the appearance of the essential organs and to search for any signs of toxicity that may have contributed to the impending death of the mice. Changes in the shape, color, size, and texture of the liver, spleen, kidneys, lungs, and heart were carefully noted. Another sign of toxicity or inflammation was the accumulation of bodily fluids around the organs of the mice.

Data analysis

The data was analyzed using GraphPad Prism 6, a statistical software program. The findings are shown as mean $\pm$ standard error (SEM), and post hoc tests, such as the Tukey-Kramer (Tukey's) and Dunnett's multiple comparisons tests were used to assess mean differences for all groups and within treatment groups after a one-way ANOVA. Mice survival times (ST) were plotted using Kaplan-Meier Plots, and a Log-rank (Mantel-Cox) test was used to compare the outcomes between the test and control groups. $P < 0.05$ was regarded as a significant value.

RESULTS

Suppressive activity of plant extracts

The efficacy of *D. chamaethamnus* and *G. coleosperma* extracts as antimalarials, both aqueous and organic extracts, was evaluated by determining the parasite load, weight changes, and survival time for all groups of mice infected with *P. berghei*.

Parasite load

According to the results, various treatment groups for *D. chamaethamnus* had lower mean parasite counts than the vehicle group (control) ($P < 0.05$) (Figure 1). The mean parasite count for the aqueous extract was 5.94, 28.10, and 25.04% at doses of 200, 400, and 800 mgkg⁻¹, respectively, with a higher percent parasite reduction at

400 than 800 mgkg⁻¹. The parasite count was lowest at 200 mgkg⁻¹. The trend in parasitaemia was reversed for the organic extract, with parasitaemia levels of 27.28, 22.43, and 15.23% at 200, 400, and 800 mgkg⁻¹ doses. With a decreased parasite load at 400 and 800 mgkg⁻¹ ($P < 0.05$), the organic extracts demonstrated stronger antiplasmodial activity than the aqueous extracts. When compared to the vehicle group, *G. coleosperma* extracts decreased the *Plasmodium* load in infected mice by 14.35%; however, this decrease was not statistically significant ($P > 0.05$). At doses of 200, 400, and 800 mgkg⁻¹, the mean parasite counts for the aqueous extract were 20.16, 12.57, and 11.68, whereas the mean parasite counts for the organic extract were 11.05, 9.54, and 10.10 mgkg⁻¹ (Figure 2). Because the parasite count was consistent at different dosages ($P > 0.005$), there was no dose dependency for the organic extract of *G. coleosperma*. The organic extracts showed a greater parasite decrease than the aqueous extracts; however, not statistically significant ($P > 0.05$). Furthermore, increasing dosages of plant extracts did not result in a decrease in the parasite levels in mice (Table 2).

Weight changes

The changes in body weight were observed in mice treated with *D. chamaethamnus* extracts for 4- and 6 days post-infection with *P. berghei* (Figure 3). On day four, a decrease in weight associated with infection was observed for every treatment group. On Day 4, a weight increase for chloroquine was noted. The mice's weights in the treatment groups were similar to those of the vehicle control group and the mice that were given chloroquine ($P > 0.05$). On the sixth day, the mice's weight loss was more noticeable. In treatment groups, this was suggested for the organic extract at 200 mgkg⁻¹ (-22.22%), 400 mgkg⁻¹ (-20.20%), and 800 mgkg⁻¹ (-12.87%), and for the aqueous extract at 200 mgkg⁻¹ (-14.29%), 400 mgkg⁻¹ (-19.64%), and 800 mgkg⁻¹ (-17.09%). The mice treated with chloroquine lost the weight they had acquired over days five and six. The treated mice's weight changes were not statistically significant ($P > 0.05$) as compared to the vehicle (-20.67%) and chloroquine (2.04%) groups. This indicates that both the *D. chamaethamnus* extracts (treatment) and chloroquine did not prevent weight loss in mice with *P. berghei* infection. There was no dose-dependent change in body weight for the mouse groups. Additionally, comparing the groups that received the organic and aqueous extracts on days 4 and 6, there were no significant differences in weight changes ($P > 0.05$).

All groups treated with aqueous and organic extracts showed an increase in weight on day four for *G. coleosperma* (Figure 4), except GCOR400 and GCAQ800. On day 6, a decrease in weight was observed, although these changes were not statistically

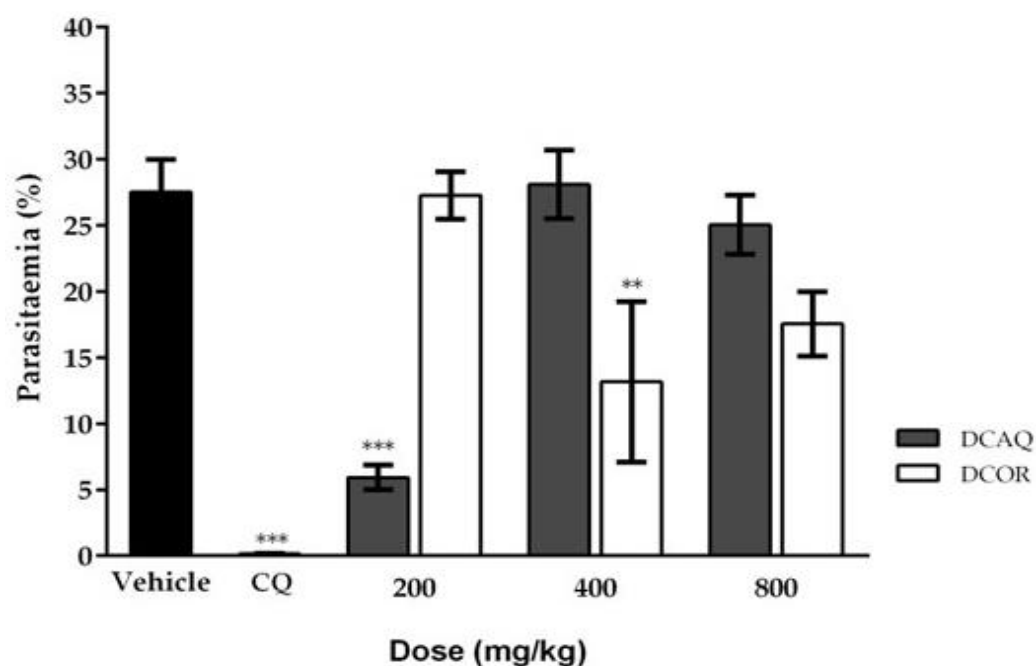


Figure 1. The antiplasmodial effects of *D. chamaethamnus* aqueous (DCAQ) and organic (DCOR) extracts on the parasite load (that is, parasitaemia) in male Swiss albino mice four days after infection in comparison to the vehicle (control group) and chloroquine (CQ). Data are presented as means $\pm$ SEM. * and ** signifies $P < 0.05$ and $P < 0.01$ simultaneously, compared to the vehicle group (one-way ANOVA followed by Dunnett's post hoc test).

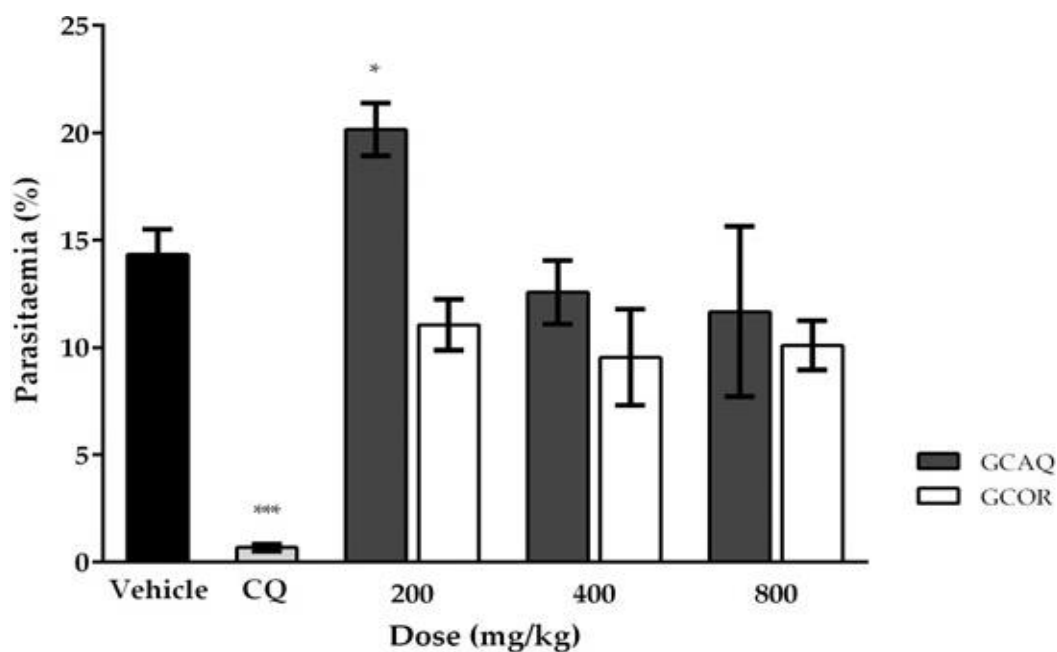
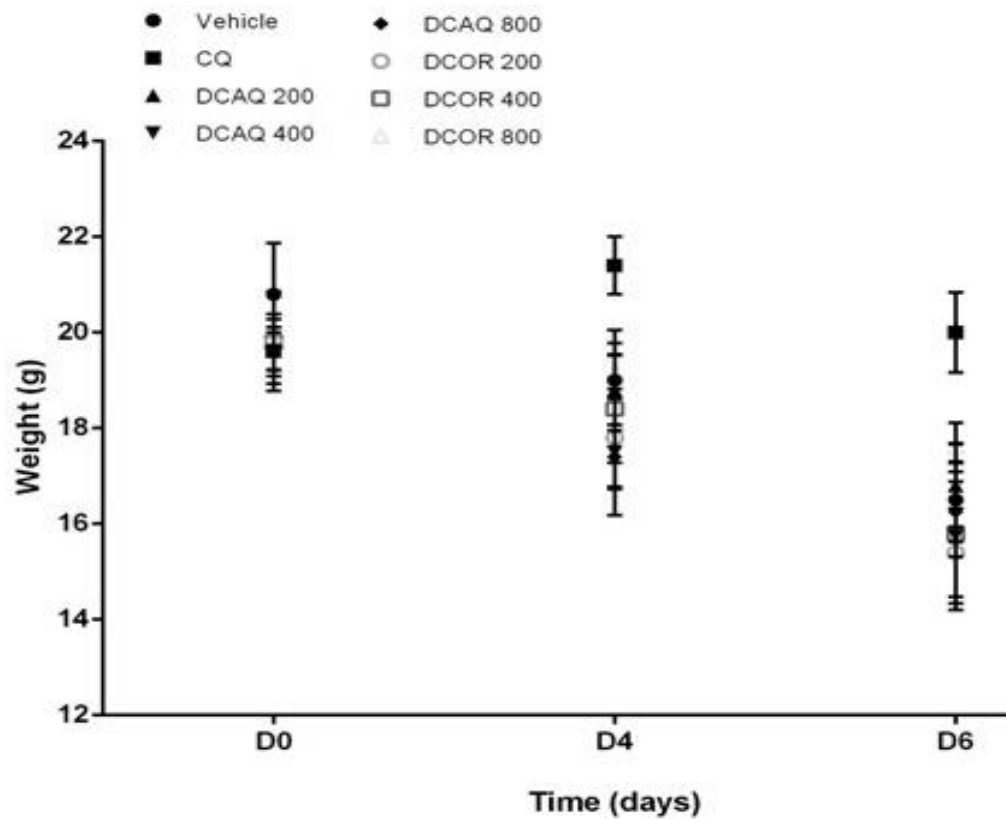


Figure 2. Antiplasmodial effects of *G. coleosperma* aqueous (GCAQ) and organic (GCOR) extracts on the parasite load (that is parasitaemia) in male Swiss albino mice four days after infection in comparison to the vehicle (control group) and chloroquine (CQ). Data are presented as means $\pm$ SEM. *, ** and *** signify $P < 0.05$, $P < 0.01$, and $P < 0.001$ simultaneously compared to the vehicle group (one-way ANOVA followed by Dunnett's post hoc test).

Table 2. Summary of percentage growth inhibition of *Plasmodia* parasites exhibited by aqueous and organic root extracts of *D. chamaethamnus* and *G. coleosperma* in *P. berghei*-infected mice.

Plant	Extract	Dose (mgkg ⁻¹ , PO)	Inhibition (%)
<i>D. chamaethamnus</i>	Aqueous	200	78.44
		400	< 1.00
		800	9.01
	Organic	200	0.89
		400	18.52
		800	44.66
<i>G. coleosperma</i>	Aqueous	200	< 1.00
		400	12.37
		800	18.61
	Organic	200	23.00
		400	33.48
		800	29.59
Chloroquine	-	25	99.37
Vehicle	-	0	0.00

**Figure 3.** Effects of *D. chamaethamnus* aqueous and organic extracts on weight in *Plasmodium berghei*-infected mice on Days 4 and 6 after infection in comparison to vehicle (control) and chloroquine (CQ: standard).

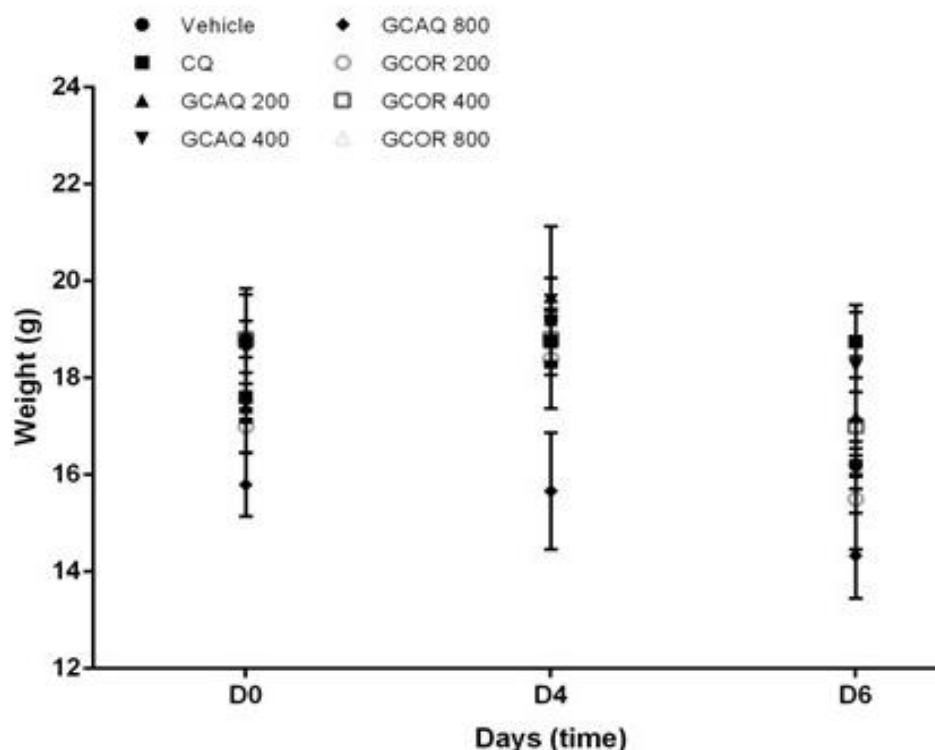


Figure 4. Effects of *G. coleosperma* aqueous and organic extracts on weight in *Plasmodium berghei*-infected mice on Days 4 and 6 after infection in comparison to vehicle (control) and chloroquine (CQ: standard).

significant ($P > 0.05$). There was a weight reduction of the treatment groups on day 6 for GCAQ at 200 mgkg^{-1} (-1.15%), 400 mgkg^{-1} (-1.88%), and 800 mgkg^{-1} (-9.30%); and for GCOR at 200 mgkg^{-1} (-8.82%), 400 mgkg^{-1} (-9.57%) and 800 mgkg^{-1} (-10.00%). By day six, mice treated with chloroquine showed no change in weight increase from day four. The differences in body weight between the extracts and between the extracts and chloroquine were not statistically significant, nor were these changes dose-dependent. These and chloroquine-treated mice's weight fluctuations on days 4 and 6 were similar to those of the vehicle control group (-13.83%) ($P > 0.05$). Weight loss was not inhibited by extracts of *G. coleosperma* or chloroquine (Table 3).

Survival time

Extracts from *D. chamaethamnus* were able to improve the survival of the mice with malaria in every group (Figure 5), and this difference was statistically significant when compared to the negative control ($P < 0.05$). The control group (vehicle) that received no treatment had the lowest median survival time (MST) of 12 days, while the

MST for the chloroquine group was not determined since three of the five mice were still alive on day 29, the last day of the observation period. The groups that received 200, 400, and 800 mgkg^{-1} of the organic extract had MSTs of 13, 16, and 18 days, respectively, whereas the groups that received 200, 400, and 800 mgkg^{-1} of the aqueous extract had MSTs of 17, 12.5, and 13 days. Mice with a confirmed malaria infection fared better when given extracts from *G. coleosperma* (Figure 6). When compared to the negative control, the test groups' survival times were statistically significant ($P < 0.05$). The MST was 12 days for the vehicle control group, 16 days for the groups that received 200 and 400 mgkg^{-1} of the organic extract, and 19 days for the 800 mgkg^{-1} group. The groups that received 200, 400, and 800 mgkg^{-1} of the aqueous extract, respectively, had MSTs of 17, 14, and 15 days. Overall, mice treated with the highest dose of the organic extract of both *D. chamaethamnus* and *G. coleosperma* had increased survival times, but mice treated with the highest dose of the aqueous extract had shorter survival times. However, these differences were comparable ($P > 0.05$). The MST for the aqueous and organic extracts of *D. chamaethamnus* and *G. coleosperma* also showed no significant differences ($P > 0.05$) (Table 4).

Table 3. Summary of the weight changes in malaria-infected mice, treated with *D. chamaethamnus* and *G. coleosperma* extracts, over 4 and 6 days compared to vehicle (control) and chloroquine (standard).

Treatment	Extract	Concentration (mg/kg)	Weight (g)			Difference in weight (%)	
			D0	D4	D6	D0-D4	D0-D6
Vehicle	-	-	20.80	19.00	16.50	-8.65	-20.67
Chloroquine	-	25	19.60	21.40	20.00	9.18	2.04
<i>D. chamaethamnus</i>	Aqueous	200	19.60	18.80	16.80	-4.08	-14.29
		400	19.60	17.50	15.75	-10.71	-19.64
		800	19.60	17.40	16.25	-11.22	-17.09
	Organic	200	19.80	17.80	15.40	-10.10	-22.22
		400	19.80	18.40	15.80	-7.07	-20.20
		800	20.20	19.20	17.60	-4.95	-12.87
<i>G. coleosperma</i>	Aqueous	200	17.40	19.20	17.20	10.34	-1.15
		400	18.60	19.60	18.25	5.38	-1.88
		800	15.80	15.67	14.33	-0.82	-9.30
	Organic	200	17.00	18.40	15.50	8.24	-8.82
		400	18.80	18.80	17.00	0	-9.57
		800	18.00	18.80	16.20	4.44	-10.00

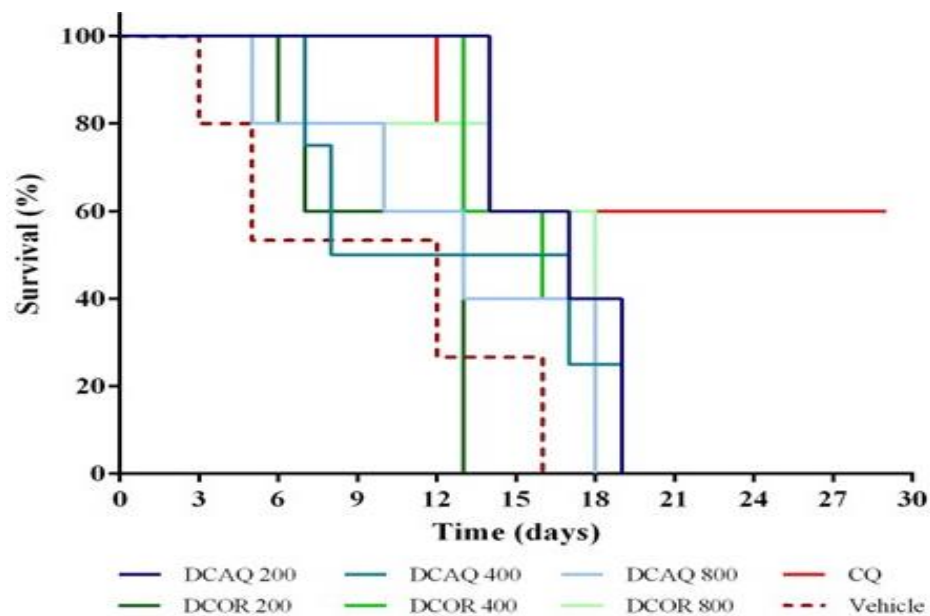


Figure 5. The survival time (ST) for mice treated with varying concentrations (200, 400, and 800 mg/kg) of *D. chamaethamnus* (DC) aqueous (AQ) and organic (OR) extracts given once daily O.P. for four days in a row is displayed in a Kaplan-Meier plot. The Log-rank (Mantel-Cox) test revealed significant differences between the test and control groups ($P = 0.0344$).

Prophylactic activity of plant extracts

The repository test or residual infection approach was used to evaluate the prophylactic efficacy of the crude

extracts of *D. chamaethamnus* and *G. coleosperma*. Investigations were limited to the aqueous extracts. This is due to the fact that there was no discernible difference in the suppressive antiparasmodial properties of the two

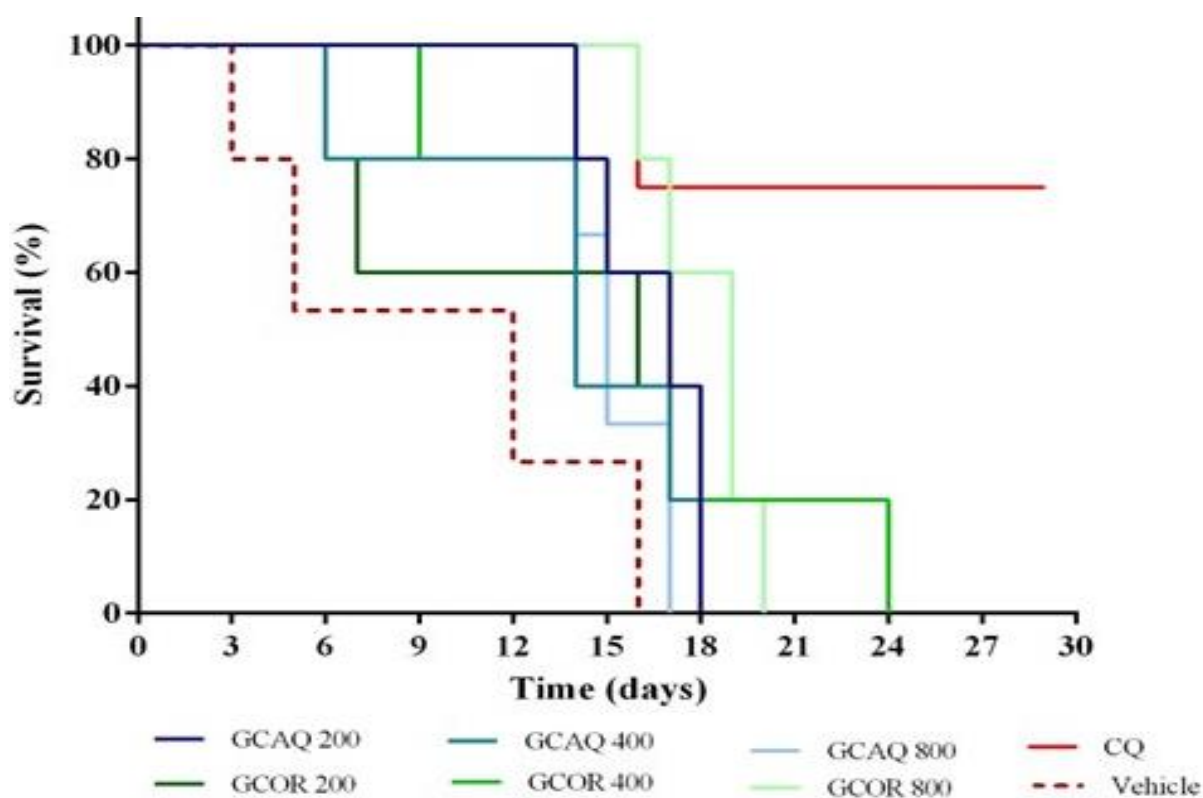


Figure 6. The survival time (ST) of mice treated with varying dosages (200, 400, and 800 mg/kg) of aqueous (AQ) and organic (OR) extracts of *G. coleosperma* (GC) given once daily P.O. for four days in a row is displayed in a Kaplan-Meier plot. The Log-rank (Mantel-Cox) test revealed significant differences between the test and control groups ($P = 0.0026$).

Table 4. Summary of the median survival time (MST) for mice subjected to different doses of aqueous and organic extracts of *D. chamaethamnus* and *G. coleosperma*.

Plant extract	Extract	Dose (mgkg ⁻¹)	MST (days)*
<i>D. chamaethamnus</i>	Aqueous	200	17
		400	12.5
		800	13
	Organic	200	13
		400	16
		800	18
<i>G. coleosperma</i>	Aqueous	200	17
		400	14
		800	15
	Organic	200	16
		400	16
		800	19
Chloroquine	-	25	Undefined
Vehicle	-	-	12

*Tabulated using GraphPad Prism 6 (Survival analyses).

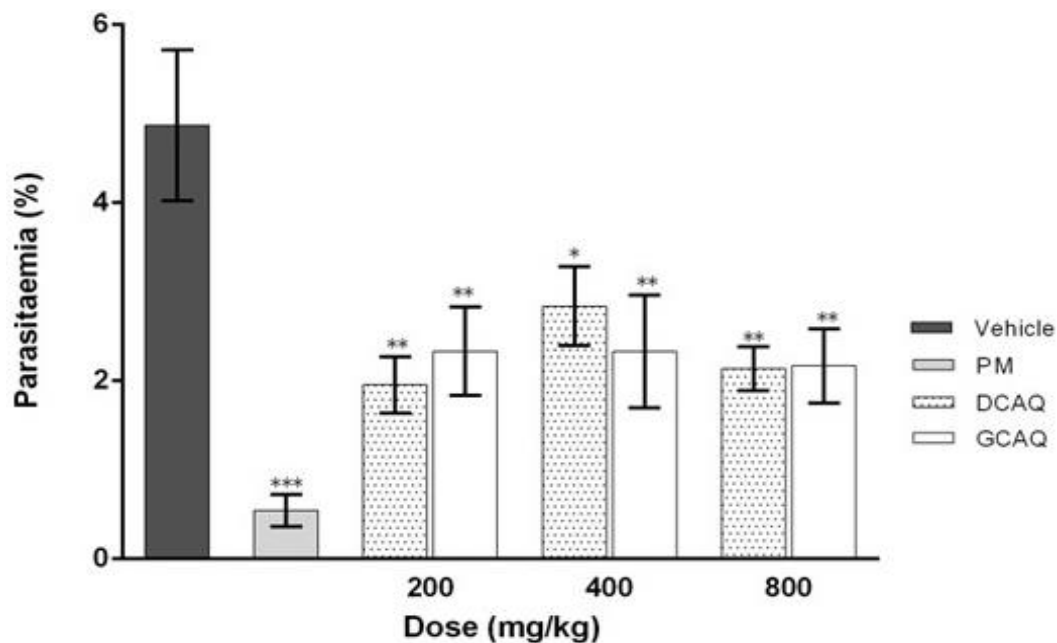


Figure 7. Prophylactic effects of *D. chamaethamnus* and *G. coleosperma* aqueous extracts on the parasite load (parasitaemia) in male Swiss albino mice 72 h after infection. The means $\pm$ SEM are used to display the data. * $P < 0.05$; ** $P < 0.01$, in comparison to the vehicle group (one-way ANOVA followed by Dunnett's post hoc test).

plants' aqueous and organic extracts; also, water is the solvent most commonly utilized in ethnomedical contexts to make herbal treatments. Furthermore, in Namibia's rural Zambezi region, the plants being studied are prepared as decoctions. All groups of mice infected with *P. berghei* had their parasite load, survival duration, and weight changes assessed as well.

Parasite load

When compared to the vehicle control group, the parasite load was lower in the aqueous extracts of *D. chamaethamnus* and *G. coleosperma* ($P < 0.05$) (Figure 7). Nonetheless, there was no significant difference in parasitaemia between the two plants (treatment groups). When the mice were given the aqueous extract of *D. chamaethamnus*, their parasite load was 1.96% at 200 mg kg^{-1} , 2.84% at 400 mg kg^{-1} , and 2.14% at 800 mg kg^{-1} . The parasite load for *G. coleosperma* was 2.17 at 800 mg kg^{-1} , 2.33 at 400 mg kg^{-1} , and 2.33 at 200 mg kg^{-1} . Given that the antiparasmodial effects of the different extract doses were similar ($P > 0.05$), neither plant's extract-induced decrease in parasite load was dose-dependent. *D. chamaethamnus* aqueous extract demonstrated antiparasmodial activities of 50.96, 41.68, and 56.13% at 200, 400, and 800 mg kg^{-1} /day, respectively; additionally, the lowest dose (200 mg kg^{-1}) had the highest activity, followed by the highest dose

(800 mg kg^{-1}), and the lowest activity was 400 mg kg^{-1} . At a dose of 10 mg kg^{-1} , the positive control, pyrimethamine, exhibited an 88.79% growth inhibition percentage. Overall, both plants' aqueous extracts exhibited similar antiparasmodial effects at all doses ($P > 0.05$).

Weight changes

On day 4, a decrease in body weight due to infection with malaria was seen in all mice in the aqueous extracts of both *D. chamaethamnus* and *G. coleosperma* (Figure 8). No weight loss was noted in GCAQ200 (1.9%). However, on day 7, the weights of DCAQ200 (1.0%), GCAQ200 (5.6%), GCAQ400 (1.0%), and GCAQ800 (2.6%) increased. When compared to the controls (pyrimethamine and vehicle), these changes were not statistically significant ($P > 0.05$). Additionally, weight changes for both Days 4 and 7 did not reveal any significant differences in the extracts' doses or between *D. chamaethamnus* and *G. coleosperma* extracts ($P > 0.05$).

Survival time

When the survival times of the mice in the experimental groups and the control group (vehicle) were compared (Figure 9), the results showed statistical significance ($P <$

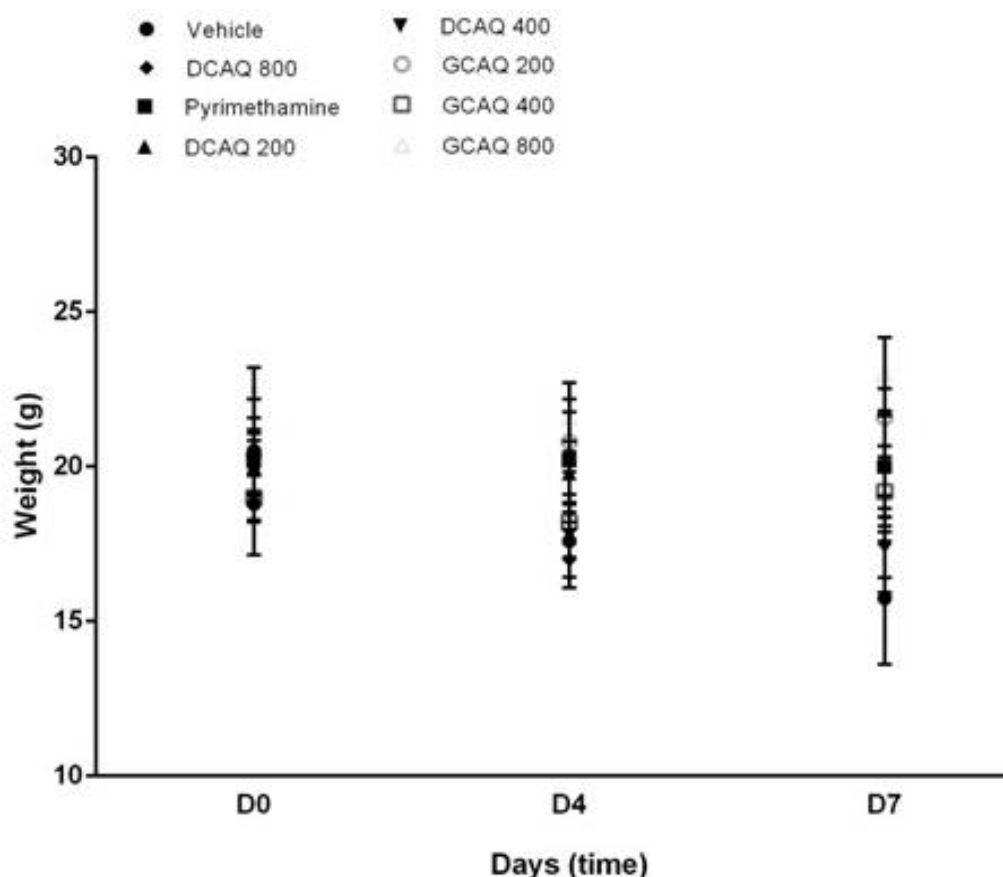


Figure 8. Prophylactic effects of *D. chamaethamnus* and *G. coleosperma* aqueous extracts on weight in *Plasmodium berghei*-infected mice on Days 4 and 7 in comparison to pyrimethamine (standard) and vehicle (control). According to Tukey's multiple comparisons test, there was no significant difference between the treatment groups ($P > 0.005$).

0.05). While no dose-dependent impact was seen, the mice treated with all dosages of the aqueous extracts for both *D. chamaethamnus* and *G. coleosperma* survived longer than those in the vehicle control group, according to the data. Mice given the highest dose of the aqueous extract of both *D. chamaethamnus* and *G. coleosperma* had a greater rate of survival. Nonetheless, the mice's median survival times between these groups were comparable ($P > 0.05$). For DCAQ and GCAQ, the highest dose (800 mg kg^{-1}) produced the longest survival times, 23 and 11 days, respectively. The MST of the mice was 13 days for the group that received pyrimethamine, 9 days for the control group (vehicle), and 17 days for the groups that received 200 and 400 mg kg^{-1} of DCAQ and 10 days for the two groups that received 200 and 400 mg kg^{-1} of GCAQ, respectively. Additionally, the mice treated with *D. chamaethamnus* aqueous extracts had a longer survival time than the mice treated with *G. coleosperma* aqueous extracts, and they also outlived the mice treated with pyrimethamine ($P > 0.05$).

Acute oral toxicity evaluation

Clinical observations

The safety of the plant extracts was assessed in healthy female mice using clinical indicators of toxicity, including a rough coat of hair (hair erection), vomiting, diarrhea, foaming in the mouth, incoordination of gait, difficulty breathing (dyspnea), unprovoked behavior (that is, fighting or biting), alertness, restlessness, and clonic convulsions (jerking), lethargy or recumbency, and coma. Observations showed that mice given aqueous and organic extracts of both *D. chamaethamnus* and *G. coleosperma* at doses up to 2000 mg/kg did not exhibit any behavioural changes 24 h after PO treatment or over the 14-day study period (Table 5). Table 5 also displays the number of mice that were still alive at the end of the study period. The median lethal dosage (LD_{50}) was more than 5000 mg kg^{-1} , as no deaths were reported at any of the levels. Each mouse underwent a macroscopical gross

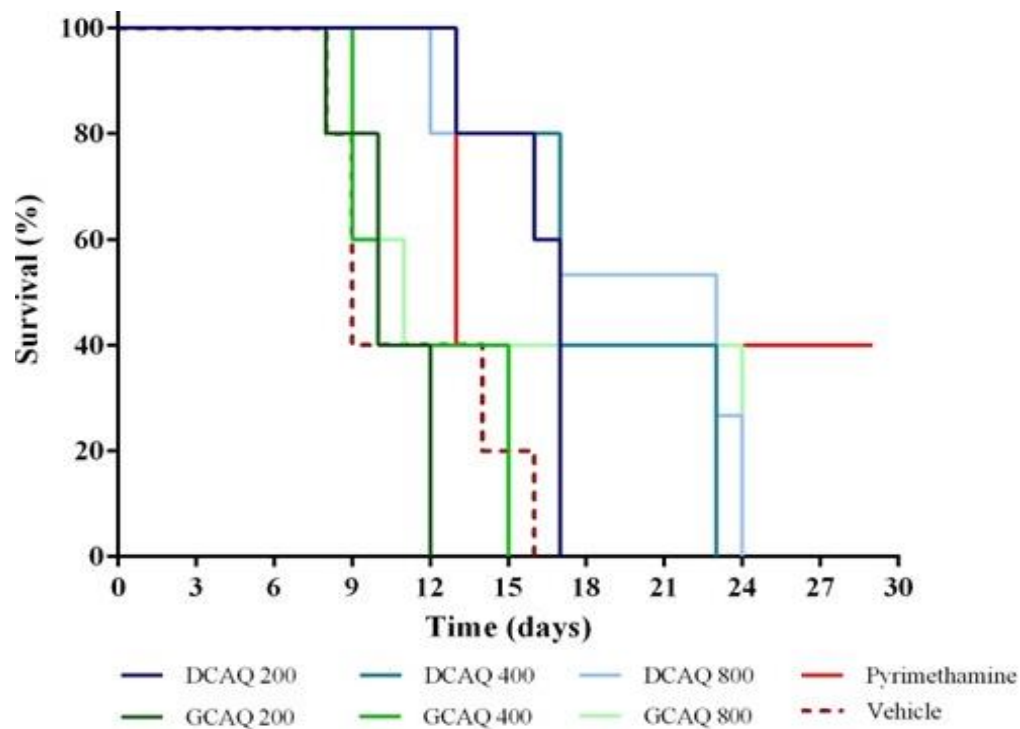


Figure 9. The survival time (ST) of mice treated with varying dosages (200, 400, and 800 mg/kg) of aqueous extracts (AQ) of *D. chamaethamnus* (DC) and *G. coleosperma* (GC) given once daily P.O. for four days in a row is displayed in a Kaplan-Meier plot. According to the Log-rank (Mantel-Cox) test, the difference between the test and control groups was significant at $P < 0.005$.

Table 5. Acute oral toxicity in healthy female mice caused by the aqueous and organic extracts of *D. chamaethamnus* and *G. coleosperma*.

Treatment	Extract	Dose (mgkg ⁻¹)	No. of mice	No. of dead mice	Clinical signs
<i>D. chamaethamnus</i>	Aqueous	300	3	0	None
		300*	3	0	None
		2000	3	0	None
		2000*	3	0	None
	Organic	300	3	0	None
		300*	3	0	None
		2000	3	0	None
		2000*	3	0	None
<i>G. coleosperma</i>	Aqueous	300	3	0	None
		300*	3	0	None
		2000	3	0	None
		2000*	3	0	None
	Organic	300	3	0	None
		300*	3	0	None
		2000	3	0	None
		2000*	3	0	None

*A repeat of the dose to a second group of mice, when 0 or 1 died in the previous group.

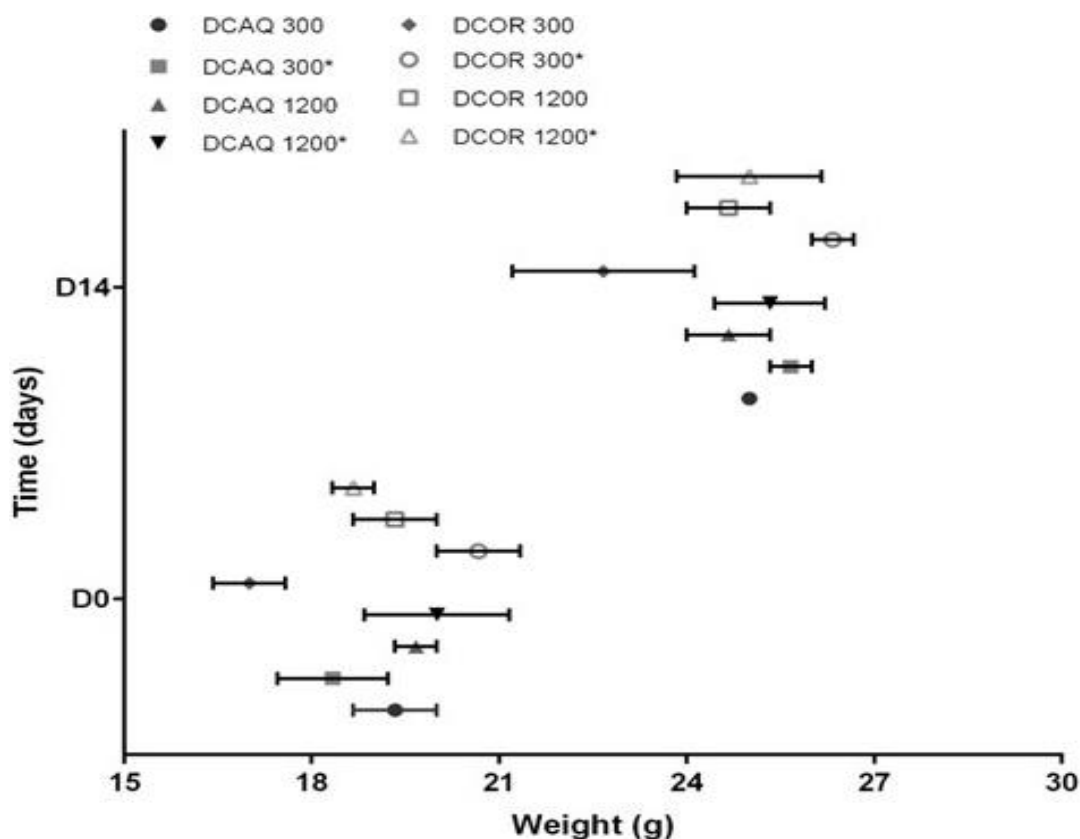


Figure 10. Weight changes in mice during 14 days for determining acute oral toxicity after *D. chamaethamnus* (DC) aqueous (AQ) and organic (OR) extracts were administered at fixed dosages of 300 and 2000 mg/kg. Between D0 and D14, the dosed groups' values change considerably ($P < 0.05$). *When 0 or 1 mice in the first group die, the dose is repeated to a second set of mice.

post-mortem examination after the study, in addition to clinical observations. No obvious abnormalities of the heart, spleen, liver, kidneys, or lungs were found. The organs' size and colour changes, hemorrhagic lesions, enteritis and congestion were among the abnormalities.

Weight changes

Figures 10 and 11 present the change in body weight for each group of mice, which were weighed at the beginning and end of the experiment. During the 14-day period, no reduction in body weight was seen in any of the animals from the treatment groups (300, 300*, and 2000, 2000* mg/kg of aqueous and organic extracts of *D. chamaethamnus* and *G. coleosperma*); on the contrary, a substantial increase in body weight was seen ($P < 0.05$). However, within a treatment group, there was no significant difference in weight increase across various doses. The group that received *G. coleosperma* extracts noted a lower percentage of weight gain than the *D. chamaethamnus* group. Additionally, the group that

received a 2000 mg/kg dose of extracts had a lower percentage weight increase than the group that received 300 mg/kg. In mice, weight gain was lowest for 2000* mg/kg with a 6.1% increase and highest for 300 mg/kg with a 19.3% increase in GCAQ, while it was lowest for 2000 mg/kg with a 26.7% increase and highest for 300 mg/kg with a 39.1% increase in GCOR; in GCOR, the highest weight increase was for 300 mg/kg (33.7%) and the least for 2000 mg/kg (21.7%). In contrast, there was inconsistent weight increase across concentrations in DCOR. All mice, excluding those receiving GCAQ, showed an overall weight gain of greater than 20%.

DISCUSSION

Traditional plant-based medicines are widely recognized and accepted, yet they are not incorporated into mainstream healthcare policies in some countries, including Namibia. Validating their use provides information that can help inform researchers and policymakers. For this study, plants traditionally used to

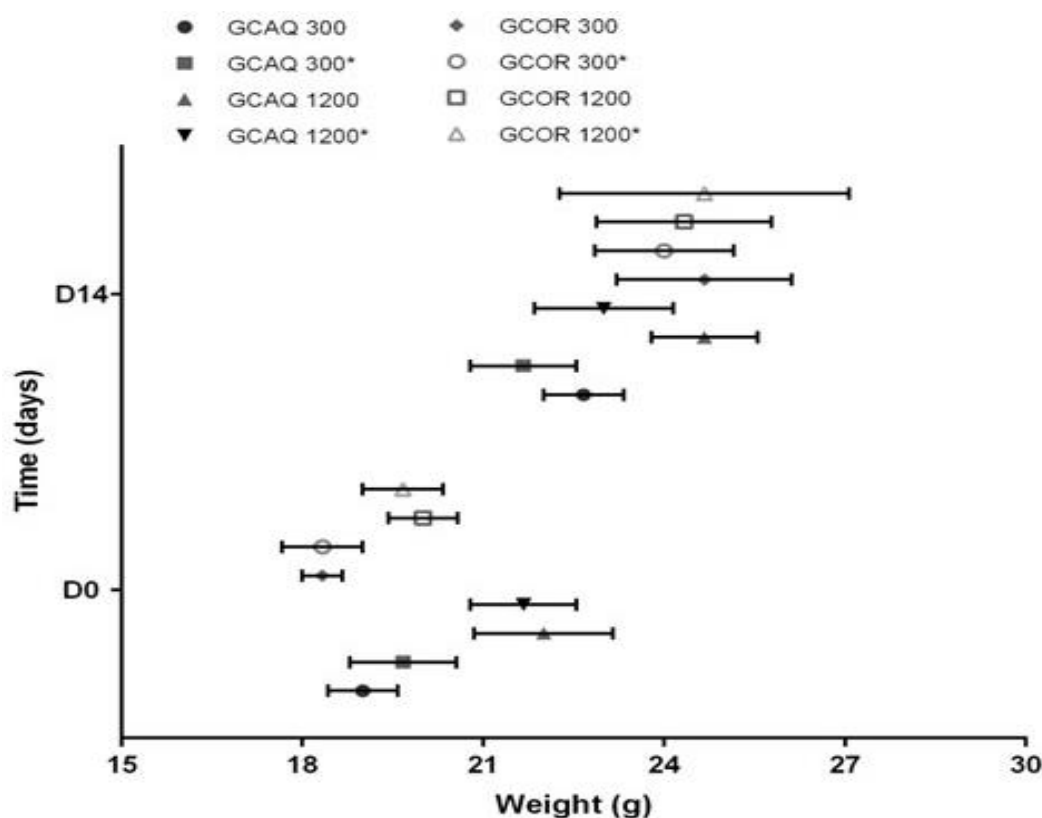


Figure 11. Weight changes in mice during a 14-day period for determining acute oral toxicity after *G. coleosperma* (GC) aqueous (AQ) and organic (OR) extracts were administered at fixed dosages of 300 and 2000 mg/kg. There is a significant difference in the dose group values between D0 and D14 ($P < 0.05$). *A second set of mice is given the same dose after either 0 or 1 of the first group dies.

treat malaria were evaluated for their antiparasitic activity and toxicity in a small animal model. Animal studies inform human research by addressing significant gaps and providing insight into disease processes and biological pathways (Hartung, 2024). However, using animal models in drug discovery has its limitations – they sometimes do not predict an accurate human response, rendering it difficult to identify medications that are effective and or harmful (Hartung, 2024). A small animal model was used because it has been validated through the testing of various conventional antimalarials used in humans, including chloroquine, halofantrine, mefloquine, and, more recently, artemisinin derivatives (Nafiu et al., 2013), and it can simulate the progression, diagnosis and treatment of the disease in humans (Mukherjee et al., 2022). Due to the wide range of complex chemicals found in plants and the common use of herbal treatments in traditional settings, crude plant extracts were investigated. The effectiveness of these treatments is influenced by the additive or synergistic interactions between plant metabolites (Vaou et al., 2022). Furthermore, increased activity, as well as delaying the

onset of resistance in pathogenic organisms, has been associated with these types of interactions (Vaou et al., 2022).

The antiparasitic activity of *D. chamaethamnus* and *G. coleosperma* against the *Plasmodia* parasites (*P. berghei*) was evaluated by determining mean parasitaemia and percentage growth inhibition of parasites, as it is the most reliable measure for determining antimalarial activity (Nafiu et al., 2013). The study's findings demonstrated that the two plants' extracts suppressed the growth of the *Plasmodia* parasites. There was a reduction in the number of parasitized erythrocytes observed for the aqueous and organic extracts of both plants compared to the negative control (that is no treatment). The aqueous extract of *D. chamaethamnus* at 200 mgkg⁻¹ (that is the lowest dose), exhibited higher activity (78.44%) than the to the higher doses 400 and 800 mgkg⁻¹. This could be because the aqueous extract, especially at higher doses, could not completely dissolve in the solvent, which may have affected the doses administered; hence, a dose-dependent effect was not observed. There are no

previous studies to support the *in vivo* antimalarial activity of these plants. However, *in vitro* activity has been reported for these two plants (Du Preez et al., 2020; du Preez-Bruwer et al., 2022) and related species (Titanji et al., 2008; Prachayasittikul et al., 2010; Nafuka, 2014) which supports the observed antiplasmodial activities in this study.

Various compounds known for their antiplasmodial activity have been identified, including alkaloids (Bitombo et al., 2021), terpenoids (Amoa Onguéné et al., 2013), steroids, coumarins, anthraquinones, and flavonoids (Bekono et al., 2020). Previous studies have shown that the plants under investigation contain alkaloids, anthraquinones, flavonoids, steroids and terpenoids (Dushimemaria et al., 2017), and specific compounds that are known to have antimalarial properties, such as lupeol, palmitic acid, stigmasterol, and pyrogallol (du Preez-Bruwer et al., 2022), and oleic acid, α -amyrin, and squalene, and compounds such as alkanes and fatty acids (Du Preez et al., 2020). The presence of these compounds in both plant extracts may further support the findings of this study. Potential mechanisms of action for antiplasmodial activity may be attributed to the phytochemical constituents present in this plant. Alkaloids found in plants have been shown to prevent heme from being converted into hemozoin within the parasite's vacuole. Additionally, these alkaloids induce changes in the parasite's DNA, thereby inhibiting both DNA replication and transcription (Parvatkar et al., 2024). Terpenoids, including sesquiterpenes like artemisinin, function by breaking down the endoperoxide bridge of heme, which releases free radicals that cause the alkylation of malaria proteins, subsequently killing the parasites (Pandey and Tripathi, 2014). Flavonoids, however, disrupt the base pairing of nucleic acids of the *Plasmodium* parasites (Akintola et al., 2022). Furthermore, flavonoids such as silymarin prevent the production of hemozoin by binding to heme released during haemoglobin digestion (Mina et al., 2020).

The longer survival time of mice treated with both the aqueous and organic extracts of the tested plants provides more support that these plants could be potential therapeutic options for malaria. Despite the fact that the plants did not completely inhibit the growth of the parasites, their antipyretic and analgesic properties may have had a significant influence (Wanyoike et al., 2004) as demonstrated by the plants' traditional use and the presence of antioxidants and compounds with immunoregulatory properties in their extracts (Dushimemaria et al., 2017; Du Preez et al., 2020). Furthermore, the aqueous root extracts of both *D. chamaethamnus* (59.86%) and *G. coleosperma* (52.16%) showed strong preventive antiplasmodial action with a fifty percent reduction in parasite load at a dose of 200 mg/kg⁻¹ (Deharo et al., 2001). Similar results were reported in a study that showed prophylactic activities of

plants against *P. berghei* in that range (Alli et al., 2016). The mice that received all doses of the aqueous extracts for *G. coleosperma* and *D. chamaethamnus* survived longer than the animals in the vehicle control group. Terpenoids may be the cause of this response in plant extracts. According to reports, this class of phytochemicals is effective against a variety of blood-stage parasites, such as trophozoites, rings, and schizonts (Boampong et al. 2015). The plant extracts' capacity to kill all erythrocytic blood forms of the parasites makes them ideal candidates for prophylaxis agents (Castelli et al., 2010).

Furthermore, comparable antiplasmodial activities were found in the organic and aqueous extracts of each plant, indicating that the active phytoconstituents that cause the observed antimalarial effects are present in both the water and methanol-dichloromethane (1:1 v/v) extracts. This is confirmed by research comparing solvents, which shows that water had good extraction efficiency and biological activity, especially when microwave assisted, even though methanol, ethanol and acetone had higher values (Borges et al., 2020). The reasons for using water as an extractant include its accessibility, cost, and safety (Lajoie et al., 2022) and the potential to extract compounds with antimalarial properties (Phuwajaroanpong et al., 2022). Additionally, water can extract a wider range of compounds, including polar molecules (Abubakar and Haque, 2020), that may contribute to the overall antiplasmodial activity. Aqueous extracts are popular in traditional medicine (Bommakanti et al., 2023) due to their perceived lack of toxicity and ease of preparation (Plaskova and Mlcek, 2023).

To ascertain or validate the antimalarial activity of plant extracts, an investigation of the changes in body weight was conducted. The absence of malaria pathology was demonstrated by mice in all groups (vehicle, chloroquine, and treated), not losing weight four days after infection or in response to the suppressive actions of *G. coleosperma*. It's possible that the RBC parasite infection of mice hasn't advanced to the point where it is pathological. Treatment groups and control groups (vehicle and chloroquine) showed weight loss on day 6, and *D. chamaethamnus*-treated groups also showed weight loss on days 4 and 6. These findings imply that chloroquine and test plant extracts were ineffective at preventing weight loss in mice with malaria. The prophylactic test's controls and plant extracts also did not prevent weight loss. Therefore, it can be said that in this study, weight was not a reliable indication of the plant extracts' antimalarial effectiveness.

This study is the first to report *in vivo* antiplasmodial activities and toxicity, providing insights into the efficacy and safety of these plants. The findings suggest that *D. chamaethamnus* and *G. coleosperma* extracts have antiplasmodial properties and are safe. This provides a basis for these plants to be incorporated into mainstream

health care policies for the treatment of malaria and prophylaxis. The prophylactic use of the plants is new knowledge that should be shared with indigenous communities to enhance their medicinal value. The findings also highlight the significance of water as an essential base or solvent for herbal remedies. More work needs to be done, specifically on the standardization and quality control of these herbal medicines, and to test the plants in combination therapies, as they are traditionally used. Combination therapies are believed to be more effective than single treatments (Otieno et al., 2008). Other possible tests to consider include assessing antipyretic and analgesic effects and immunoregulatory properties.

Conclusion

The findings of the study demonstrate antiplasmodial activities of the extracts from *Diospyros chamaethamnus* and *G. coleosperma* by suppressing parasitaemia levels and prolonging the survival of the *Swiss Albino* mice. Additionally, the extracts were also non-toxic. Therefore, the results, confirm the ethnomedicinal use of these plants in treating malaria and suggest that they may also serve as a preventive measure against malaria. Future research should promote the screening of *D. chamaethamnus* and *G. coleosperma* for their antiplasmodial properties, particularly in combination with other plants as discussed in this article.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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