Evaluation of Antimalarial Activity of 80% Methanolic Root Extract of *Dorstenia barnimiana* Against *Plasmodium berghei*–Infected Mice

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**Background:** Due to resistance of parasites, costs, and safety issues with currently available drugs, there is a need to discover new antimalarials. Medicinal plants are one of the most important sources of new drugs. The purpose of this study was to evaluate the antimalarial activity of a methanolic root extract of *Dorstenia barnimiana* in Swiss albino mice infected with *Plasmodium berghei*.

**Methods:** Four-day suppressive, curative, and prophylactic tests were performed on mice infected with *P. berghei* to evaluate the antimalarial activity of a methanolic root extract of the plant. Parasitemia suppression, survival time, body-weight change, rectal temperature change, and packed-cell volume were used to evaluate the activity of the extract. Data were analyzed using one-way ANOVA followed by Tukey’s post hoc test for comparisons between and within groups, with $P<0.05$ considered statistically significant.

**Results:** The *D. barnimiana* root extract showed significant ($P<0.01$) parasitemia-suppressive activities in all models compared to the negative control. The extract increased mean survival time and prevented weight loss, reduction in temperature, and anemia significantly in all tested doses in 4-day suppressive and curative tests.

**Conclusion:** Based on these findings, *D. barnimiana* root has promising antimalarial activity and can be considered a potential source to develop new agents.

**Keywords:** antimalarial, *Dorstenia barnimiana*, *Plasmodium berghei*, 4-day suppressive test, curative test, prophylactic test

**Background**

Malaria is still a major public-health problem and is one of the major obstacles to the socioeconomic development of several developing countries. According to the 2020 World Malaria Report, in 2019 alone, malaria caused 229 million morbidities and 409,000 deaths globally. A majority of the deaths and morbidities were in Africa. In 2019, 94% of malaria morbidity and 94% of malaria mortality was in Africa. Children aged <5 years are the most affected age-group, accounting for 67% of all mortality due to malaria globally in 2019 alone.

In Ethiopia, like other African countries, malaria is one of the leading causes of morbidity and mortality. Nearly 80% of districts in Ethiopia are considered “malarious,” and around 68% of the population are at risk of malaria. As a result of the unstable transmission of malaria in Ethiopia throughout the year, protective immunity among populations is relatively low. Among the *Plasmodium* spp.,
P. falciparum is the major cause of malaria in Ethiopia, responsible for 65%–75% of cases, with P. vivax responsible for 25%–35%. P. ovale and P. malariae are rare.4–8

Due to the unavailability of an effective vaccine, chemotherapy and vector control comprise the core approach to combat the devastating effect of malaria. However, this modality of management is still confronted by numerous challenges, including the alarming spread of insecticide-resistant female anopheles mosquitoes, chemotherapy-resistant Plasmodium spp. in many parts of the world, and a limited number of effective antimalarial drugs.10–14 Therefore, new knowledge, products, and measures, and in particular new antimalarial drugs, are urgently needed to confront the devastating effect of malaria.

Traditionally used medicinal plants are a relevant source of new therapeutic approaches. According to the World Health Organization, approximately 80% of African and Asian populations depend on traditional medicine for their primary health-care needs. In Ethiopia, >80% of the population depends on traditional medicine, mainly medicinal plants, to treat a variety of diseases, including malaria, to meet their primary health-care needs. This is due to the cultural acceptability of healers, the relatively low cost, and difficulty in accessing modern medicine.15,16 Medicinal plants are the chief source of drugs for medicine. Approximately 25% of currently prescribed drugs are obtained from plants, and >11% of essential drugs are obtained from plants or synthetic drugs obtained from natural precursors.17

Globally, >1,000 plant species have been reported to be used for the treatment of malaria traditionally, and thus are potential sources of new antimalarial drugs.18 The root of Dorstenia barnimiana, known locally as “werk bemieda”, is an erect perennial herb, and one of the most frequently used plants for medicinal purposes. It has been used by the local community as a traditional remedy against acute coughing (tuberculosis), leprosy, stomach illness, fungal disease, diarrhea, fever, headache, malaria, and the common cold and as an insect repellent.19–24 Specifically for malaria, the root powder is mixed with aguat in local name and fresh filtrate taken on an empty stomach once every other day for 3 days. However, there is no literature that gives scientific validation of the antimalarial activity of the root of this plant. This study was done to evaluate the antimalarial activity of the plant scientifically.

Methods

Plant Collection and Preparation of Plant Materials

The roots of D. barnimiana were collected in January 2018 from around Bahir Dar. Bahir Dar is located at the exit of Abbay from Lake Tana at an altitude of 1820 m above sea level. The city is located approximately 578 km northwest of Addis Ababa. Bahir Dar has a borderline–tropical savanna climate. Afternoon temperatures are very warm to hot year-round and morning temperatures cool; however, the diurnal range is much larger in the largely cloudless dry season. The plant was authenticated by the National Herbarium of Addis Ababa University, with voucher identity of DD003/2018 given and deposited. The fresh roots were thoroughly washed with water to and then dried under shade with optimal ventilation. After being chopped, roots were powdered using electronic crushers. The powder was kept in a well-closed amber-colored bottle at room temperature until extracted.

Preparation of Plant Extracts

Weighed using a sensitive digital balance, 100 g dried-root powder was mixed with 80% methanol in an Erlenmeyer flask at room temperature. Then, the plant and solvent mixtures were extracted by cold maceration by placing them on an orbital shaker for 3 days at 160 rpm. On the third day, the extract was separated from the marc by filtration using Whatman filter paper number 1 with pore size 0.7µm. This procedure was done three times for exclusive extraction. Then, the extract was dried by removing the methanol under reduced pressure using a rotary evaporator at 45 rpm and 40°C. The extract was further concentrated with lyophillizer (Wagtech Jouan Nordic DK-3450) at ~40°C under vacuum pressure. Finally, the percentage yield of the extract was calculated by weighing the dry extract, and all the dry extracts were stored in airtight plastic containers and maintained in a refrigerator at ~20°C until use.

Reagents and Drugs

Chloroquine phosphate, distilled water, Giemsa stain, trisodium citrate, absolute methanol, and other chemicals for phytochemical screening, such as 20% NaOH, chloroform, sulfuric acid, 95% ethanol, ferric chloride, glacial acetic acid, acetic acid Hcl, 10% ammonia, and Mayer’s reagent.
were used in this experiment. Most these were obtained from the Pharmacy Department of Bahir Dar University, and the rest purchased from the Ethiopian Pharmaceutical Supply Agency or certified suppliers.

Experimental Animals
For this experiment, 95 Swiss albino mice of both sexes aged 4–6 weeks and weighing 22–28 g were used. They were bred in the animal house of the Pharmacy Department and kept in regular plastic cages with wood-chip bedding at a controlled room temperature of (21°C ±2°C), with a 12/12-hour light/dark cycle and relative humidity of 40%–50%. They were fed pellets and drinking water ad libitum. Care and handling of the animals were in accordance with the guidelines of the Organization for Economic Cooperation and Development (OECD) and the Institute for Laboratory Animal Research. Acclimatization to a working environment was done a week before commencing the experiment.25,26

Parasite
The chloroquine-sensitive Plasmodium berghei ANKA strain, the parasite used to infect mice in all models, was purchased from the Aklilu Lemma Institute of Pathobiology. Serial blood passage from infected mouse to naive mouse weekly was done to maintain the P. berghei in our laboratory. Donor mice with a parasitemia level of 20%–30% were killed and infected blood collected by cardiac puncture into heparinized vacutainer tubes containing 0.5% trisodium citrate. The blood was then diluted in normal saline based on the parasitemia level of the donor mouse and 0.2mL infected blood containing about 10^7 P. berghei (quantified with thick blood film) parasitized red blood cells (RBCs) transferred to uninfected mice intraperitonally (IP). Each mouse used in the study was infected in the same way.27

Preliminary Phytochemical Screening
The presence or absence of alkaloids, flavonoids, terpenoids, phenols, saponins, tannins, cardiac glycosides, phytosterol, and resins was tested in the methanolic root extract of D. barnimiana based on established standards28,29 to relate the secondary metabolites to their antimalarial activity.

Acute Oral Toxicity Test
Acute oral toxicity of the extract was assessed in young, nulliparous, and nonpregnant Swiss albino mice based on the limit-test advice of OECD guideline 425.25 After acclimatizing for 1 week, one mouse was fasted for 4 hours, received a single oral dose of 2,000 mg/kg extract, then fasted again for the next 2 hours. The mouse was observed closely for the first 30 minutes after dosing and regularly for the next 24 hours, then daily for 14 days for any physical or behavioral abnormality. Based on the results from the first mouse, an additional four mice were fasted overnight, administered a single dose of 2,000 mg/kg, and followed similarly.

Grouping and Dosing of Animals
Thirty P. berghei–infected mice were randomly divided into five groups (n=6) in each model to evaluate the antimalarial activity of the extract. Group 1 was used as a negative control and received 10 mL/kg (0.2–0.3 mL) distilled water, groups 2 –4 received extracts of D. barnimiana 100 mg/kg (DB100), DB200, and DB400, respectively, and group 5 was used as positive control, receiving chloroquine 25 mg/kg (CQ25). All treatments were given by oral gavage.

In Vivo Antimalarial Screening
Four-Day Suppressive Test
This test followed a standard protocol.30,31 Three hours after P. berghei infection, treatment commenced on the first day and continued for the next 3 days once daily (D0–D3). Parasitemia level and percentage parasitemia suppression were determined by taking blood from the tail of each mouse on the fifth day (D4).27 Each mouse was also observed for 28 days after treatment to determine mean survival time. Weight, temperature, and packed-cell volume (PCV) were determined just before infection and on the fifth day (D4).

Curative Test
This test was done using the method described by Ryley and Peters.32 Thirty adult Swiss albino mice of both sexes were infected with P. berghei on the first day (D0). Treatment was commenced after 72 hours’ (D3) infection with P. berghei, then given once daily from day 3 (D3) to day 6 (D6). Parasitemia levels and percentage parasitemia suppression were determined by taking blood from the tail of each mouse on the seventh day. Each mouse was observed till 28 days postinfection to determine mean survival time. Rectal temperature, body weight, and PCV were measured on day 3 (before treatment) and day 7 (after treatment).
Prophylactic Test
Evaluation of the prophylactic potential of the extract was done as per prior methods. Thirty Swiss albino mice were treated daily for four (D0–D3) consecutive days. Each mouse was infected with P. berghei IP on the fifth day (D4). Parasitemia levels and percentage parasitemia suppression were determined by taking blood from the tail of each mouse 72 hours after infection (on the seventh day). Each mouse was also observed till 28 days from the initiation of treatment to determine mean survival time. Rectal temperature, body weight, and PCV were measured on day one (before treatment) and on day seven (last day of follow-up).

Determination of Parasitemia Levels and Parasitemia Suppression
Thick and thin smears of blood were taken from the tail of each mouse. The smears were placed on microscope slides, fixed with absolute methanol for 30 seconds, and stained with 10% Giemsa stain at pH 7.2 for 15 minutes. The slides were taken out, washed gently with tap water, and dried at room temperature. The slides were examined under light microscopy to determine the percentage of parasitized RBCs. For each mouse, two stained slides were used, five fields on each slide examined, and the average taken. Average percentage parasitemia and percentage parasitemia suppression was calculated using the following formula, and extracts that had parasitemia suppression ≥30% were considered active.

\[
\frac{\text{Volume of erythrocytes in a given volume of blood}}{\text{Total blood volume}} \times 100\% = PCV
\]

Ethics
Handling of the animals was in accordance with the international guidelines for the use and maintenance of experimental animals. The ethical clearance was requested and obtained (HRTT/10/311/2019) from the research and ethics review committee of the Amhara Public Health Institute before commencing the actual work.

Data Analysis
Means ± SEM are used to express the data obtained. SPSS 20.0 was used for data analysis. To test for significance, inter- and intragroup ANOVA was performed, followed by Tukey’s HSD post hoc test. P<0.05 was considered statistically significant.

Results
Preliminary Phytochemical Screening
Alkaloids, flavonoids, phenols, saponins, tannins, and cardiac glycosides were detected on phytochemical screening, whereas terpenoids, phytosterol, resins were not. The yield of the root of D. barnimiana extract was 44.3% (w:w).

Acute Toxicity Study
Mortality was not observed at 2,000 mg/kg during observation, meaning the LD_{50} of the extract was >2,000 mg/kg. The mice did not show any physical or behavioral abnormalities, eg, diarrhea, laceration, reduction in motor and feeding activities, hair erection, or any other central nervous system disturbances, during observation.

Four-Day Suppressive Test
The extract suppressed parasitemia levels significantly (P<0.001) in a dose-dependent fashion compared with the negative control at all tested doses on the 4-day suppressive test. The extract had significant parasitemia-suppression effect at all tested doses, with the highest suppression
obtained at 400 mg/kg (54.87%), as shown in Table 1. However, the effect was lower compared to the standard drug — chloroquine (100%). The extract had significant ($P<0.05$ for lowest, $P<0.001$ for middle and highest doses) effects on survival time compared with the negative control. However, the survival effect was very low compared to chloroquine (Table 2).

**Table 1 Phytochemicals in *Dorstenia barnimiana***

| Secondary metabolites | Reagent                      | Result |
|-----------------------|------------------------------|--------|
| Alkaloids             | Mayer’s test                 | +      |
| Flavonoids            | Lead acetate test            | +      |
| Phenols               | Ferric chloride test         | +      |
| Saponins              | Foam test                    | +      |
| Tannins               | Gelatin Test                 | +      |
| Cardiac glycosides    | Keller Killian’s test        | +      |
| Terpenoids            | Copper acetate test          | –      |
| Phytosterol           | Salkowski’s test             | –      |
| Resins                | Precipitation test           | –      |

Notes: +, present; –, absent.

**Table 2 Parasitemia-Suppressive and Mean Survival-Time Effects of *Dorstenia barnimiana* Root Extract Against *Plasmodium berghei* on 4-Day Suppressive Test**

| % Parasitemia | % Suppression | Survival Time (days) |
|---------------|--------------|----------------------|
| NC            | 39.44±1.37   | 0.30±1.87            |
| DB100         | 25.32±3.17   | 35.78³±3.36a2       |
| DB200         | 21.95±1.29   | 43.35³±3.36a2       |
| DB400         | 17.80±1.33   | 54.87³±3.36a2       |
| CQ25          | 0            | 100³±3.36a2         |

Notes: Data expressed as means ± SEM (n=6). *Compared to NC, **chloroquine 25 mg/kg, ***100 mg/kg, 200 mg/kg, and 400 mg/kg extract. $P<0.05$; $P<0.01$; $P<0.001$.

**Table 3 Body-weight and Rectal Temperature Effects of *Dorstenia barnimiana* Root Extract Against *Plasmodium berghei* on 4-Day Suppressive Test**

| Weight (g) | %Change | Temperature (°C) | %Change |
|------------|---------|------------------|---------|
| D0         | D4      |                  |         |
| NC         | 24.83±2.04 | 20.95±2.42 | −15.62   | 36.02±0.45 | 40.10±0.52 | −15.33 |
| DB100      | 25.50±1.87 | 23.33±1.75 | −8.51b   | 36.10±0.59 | 34.85±0.28 | −3.33b   |
| DB200      | 25.82±1.47 | 24.33±0.21 | −5.80c   | 37.23±0.33 | 35.36±0.41 | −6.23c   |
| DB400      | 25.33±2.16 | 25.17±0.72 | −6.63³c  | 36.32±0.48 | 35.93±0.42 | −6.23³c  |
| CQ25       | 25.30±1.80 | 26.00±1.79 | 2.76³c   | 36.43±0.33 | 36.67±0.12 | 1.76³c   |

Notes: Data expressed as means ± SEM (n=6). *Compared to NC, **chloroquine 25 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg extract. $P<0.05$; $P<0.01$; $P<0.001$.

**Effect on Body Weight and Rectal Temperature in 4-Day Suppressive Test**

The extract prevented weight loss and decrease in rectal temperature associated with infection in a dose-dependent fashion ($P<0.05$) at all tested doses compared to the negative control (Table 3). However, the effect of the extract was significantly lower than the positive control.

**Effect on Packed-Cell Volume in Suppressive Test**

The extract also showed a significant ($P<0.001$) effect on prevention of PCV reduction associated with infection at all tested doses compared to the negative control. The effect on PCV was dose-dependent: highest at 400 mg/kg and lowest at 100 mg/kg at the end of treatment (Figure 1).

**Curative Test**

The curative effect of the extract was found to be statistically significant at all tested doses compared to the negative control (Table 4). The effect of parasitemia suppression was dose-dependent, with the highest effect at 400 mg/kg (58.28%), followed by 200 mg/kg (47.71%) and 100 mg/kg (35.65%). The extract also increased survival time significantly at all tested doses compared to the negative control. However, parasitemia-suppressive and survival effects in extract-treated groups were significantly lower than with standard drugs.

**Effect of Extract on Body Weight and Rectal Temperature in Curative Test**

Prevention of body-weight reduction in infected mice at all tested extract doses was statistically significant compared to the negative control (Table 5). However, there was no statistically significant difference between doses. The
extract also significantly prevented reduction in rectal temperature associated with infection at all tested doses compared to the negative control.

Table 4: Parasitemia-Suppressive and Mean Survival-Time Effects of *Dorstenia barnimiana* Root Extract Against *Plasmodium berghei* on Curative Test

| % Parasitemia | % Suppression | Survival Time (days) |
|---------------|---------------|----------------------|
| NC            | 48.25±2.00    | 6.83±0.75            |
| DB100         | 31.05±2.02    | 9.50±1.05            |
| DB200         | 26.27±1.13    | 13.17±1.47           |
| DB400         | 20.13±2.11    | 15.50±1.87           |
| CQ25          | 0             | 28±0                 |

Notes: Data expressed as means ± SEM (n=6). *Compared to NC,* chloroquine 25 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg extract. P<0.05; P<0.01; P<0.001.

Abbreviations: NC, negative control; DB100, *Dorstenia barnimiana* root extract 100 mg/kg; CQ25, chloroquine 25 mg/kg.

Figure 1 Packed cell–volume change in malaria-infected mice treated with extract on the 4-day suppression test compared with negative- and positive-control groups.

Effect of Extract on Packed-Cell Volume in Curative Test

PCV reduction associated with infection was significantly (P<0.05) prevented at all tested doses compared to the negative control. The effect on PCV was dose-dependent: highest at 400 mg/kg and lowest at 100 mg/kg at the end of treatment (Figure 2).

Prophylactic Test

All tested doses of the extract significantly (P<0.001) reduced the average parasite load compared to the negative control in a dose-dependent manner. Maximum parasitemia suppression was observed at the highest tested dose of 400 mg (58.15%), followed by 200 mg (48.32%) and 100 mg (33.86%). Survival time of the infected mice pretreated with the extract indicated that only the middle and highest doses were capable of significantly (P<0.001) prolonging survival time compared to controls. The lowest

Table 5: Body-weight and Rectal Temperature Effects of *Dorstenia barnimiana* Root Extract Against *Plasmodium berghei* on Curative Test

| Weight (g) | %Change | Temperature (°C) | %Change |
|------------|---------|-----------------|---------|
|            | D3      | D7              | D3      | D7              |         |
| NC         | 26.58±1.14 | 21.78±1.30       | -18.06  | 36.48±0.65   | 34.15±0.26       | -6.39   |
| DB100      | 27.23±0.74 | 25.15±0.92       | -7.64<sup>a</sup> | 36.58±0.18 | 34.99±0.48       | -4.59<sup>a</sup>   |
| DB200      | 26.85±1.01 | 25.68±0.99       | -4.36<sup>b</sup> | 36.52±0.64 | 35.88±0.58       | -2.12<sup>b</sup>   |
| DB400      | 26.62±0.91 | 26.40±0.72       | -0.83<sup>b</sup> | 36.57±0.36 | 36.17±0.38       | -0.41<sup>b</sup>   |
| CQ25       | 27.23±0.53 | 28.83±0.77       | 5.88<sup>a</sup> | 36.30±0.18 | 36.97±0.41       | 1.85<sup>a</sup>    |

Notes: Data expressed as means ± SEM (n=6). *Compared to NC,* chloroquine 25 mg/kg, 100 mg/kg, and 400 mg/kg extract. P<0.05; P<0.01; P<0.001.

Abbreviations: NC, negative control; DB100, *Dorstenia barnimiana* root extract 100 mg/kg; CQ25, chloroquine 25 mg/kg.
dose (100 mg/kg) did not increase survival time significantly compared to the negative control (Table 6).

Effect of Extract on Body Weight and Rectal Temperature in Prophylactic Test

Only the highest dose of extract showed a significant protective effect for body-weight reduction compared to the negative control. However, the middle and highest doses significantly prevented rectal temperature reduction compared to the negative control (Table 7).

Effect of Extract on Packed-Cell Volume in Prophylactic Test

Unlike the standard drug, the extract did not have a significant effect on PCV reduction associated with infection at any tested dose compared to the negative control (Figure 3).

Discussion

The root extract was found to be significantly effective in reducing parasitemia loads at all tested doses in all three models. The difference in parasitemia-suppressive activity of the extract among the doses was also statistically significant, indicating it was dose-dependent. Based on in vivo antimalarial activity classification, the root extract of the plant had moderate antimalarial activity in all three models. The parasitemia-suppressive effect of the plant was similar to other plant extracts used for malaria, such as

Table 6 Parasitemia-Suppressive and Mean Survival-Time Effects of Dorstenia barnimiana Root Extract Against Plasmodium berghei on Prophylactic Test

Table 7 Body-Weight and Rectal Temperature Effects of Dorstenia barnimiana Root Extract Against Plasmodium berghei on Prophylactic Test
as *Calpurnia aurea*,\(^\text{10}\) *Combretum molle*,\(^\text{38}\) *Echinops kebericho*,\(^\text{39}\) and *Brassica nigra*.\(^\text{40}\) Also, the extract was atoxic up to a dose of 2,000 mg/kg, which agrees with the WHO classification of hazardous substances.\(^\text{41}\) However, at all tested doses, this plant had significantly lower parasitemia-suppression activity than the positive control. This may have been due to the low level of active compound(s) in the extract.

Mean survival time was also used to evaluate the antimalarial activity of the extract in all three models. The extracts prolonged the survival of mice on the 4-day suppressive and curative tests at all tested doses in a dose-dependent manner, and at the middle and highest doses on the prophylactic test. The greatest effect on survival time was obtained with the dose achieving the highest parasitemia suppression, in agreement with other in vivo antimalarial studies.\(^\text{5,34,42,43}\) This indicates that the significant effect on survival time may be due to the suppression of parasitemia and reduced the overall pathological effect of the parasite in the study mice.

As with human malarial infection, *Plasmodium*-infected animals showed decreased food consumption and body weight during the experiment.\(^\text{44}\) As such, prevention of body-weight loss was used in this study to evaluate the antimalarial activity of plant extracts in all models. At all tested doses, the extract significantly prevented weight loss in a dose-dependent manner compared to the negative control on the 4-day suppression and curative tests, but not the prophylaxis test. This prevention of weight loss is in line with other similar studies.\(^\text{5,38,45,46}\) This effect may have been because the extract significantly suppressed parasitemia and thereby reduced anemia and the overall pathological effect of the parasite in the test groups and improved food consumption.

Additionally, in all tested models, the extract produced a significant effect on rectal temperature of the *Plasmodium*-infected mice at all tested doses, except at the lowest dose in the prophylactic model. This study was consistent with other in vivo antimalarial tests.\(^\text{47}\) This effect can be attributed to the parasiticidal activity of the extract resulting in the prevention of alteration in body temperature occurring due to the parasite infection. The parasite infection reduce to rectal temperature due to reduction food intake, reduce catabolic state.

The other important parameter in antimalarial studies is PCV, which is a measure of the relative mass of cells present in a sample of blood.\(^\text{48}\) The destruction of infected and uninfected RBCs, either by parasites or as a result of an immunoresponse to parasite infection or both, brings a reduction in PCV of RBCs in the blood,\(^\text{49}\) resulting in anemia,\(^\text{50}\) so any significant prevention of PCV loss by plant extracts can support their antiplasmodial activity. In this study, the extract was able to prevent the loss in PCV significantly compared to controls at all tested doses on the 4-day suppressive and curative tests, but not the prophylaxis one. Such prevention of PCV loss by plant extracts has also been reported in other in vivo antimalarial studies,\(^\text{38,42}\) and might be attributed to the parasite-inhibition effect of the extract preventing the destruction of RBCs by the parasite.

Secondary metabolites may be responsible for the antimalarial activity of *D. barnimiana*. Preliminary phytochemical screening of the extract revealed the presence
of secondary metabolites like alkaloids, flavonoids, phenols, saponins, tannins, and cardiac glycosides. This phytochemical profile of the plant is similar to that of other Dorstenia spp., eg, D. mannii.\textsuperscript{51}

The secondary metabolites, eg, alkaloids, found in the extract have been revealed to have antimalarial activity in other studies.\textsuperscript{52–54} One of the most important antimalarial drugs, quinine, is one of the alkaloid compounds isolated from plants.\textsuperscript{53} Flavonoids,\textsuperscript{54–58} phenols,\textsuperscript{59,60} glycosides,\textsuperscript{61} and tannins\textsuperscript{60} have also been reported to have antimalarial activity.

**Conclusion**
The methanolic extract of D. barnimiana root showed moderate antimalarial activity in all models at all tested doses. The extract significantly suppressed parasitemia and increased survival time in all three model. Prevention of loss in body weight, rectal temperature, and PCV was observed at all doses on the 4-day suppressive and curative tests, and at the highest dose on the prophylactic test. These results support the traditional use of this plant for malaria treatment. However, before recommendation for use, further studies should be conducted with different models to elucidate its mechanism of action and confirm its efficacy and safety profile.

**Abbreviations**
CQ25, chloroquine 25 mg/kg; OECD, Organization for Economic Cooperation and Development; PCV, packed-cell volume; RBCs, red blood cells.

**Data Sharing Statement**
All data used to support the findings of this study are available from the corresponding author upon reasonable request.

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**Author Contributions**
All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data, took part in drafting the article or revising it critically for important intellectual content, agreed to submit to the current journal, gave final approval to the version to be published, and agree to be accountable for all aspects of the work.

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