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Evaluation of Antiplasmodial Effect of Methanol Leaf Extract and Fractions of *Eucalyptus camadulensis* (Denhn) in Albino Wistar Mice

Amarachi Akachukwu[1], Moses Ikegbunam[2,3*], Emmanuel Enyi[2], Theresa Osarunwese Ifeyinwa Onochie-Igbinedion[2], Harrison Abone[2], Dorothy Ezeagwuna[4], Joy Nnanna[2], Chibueze Ihekwereme[1], Chukwuemeka Nworu[5]

[1]Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Awka, Nigeria
[2]Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Awka, Nigeria
[3]Molecular Research Foundation for Students and Scientists, Nnamdi Azikiwe University, Awka, Nigeria
[4]Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Nigeria
[5]Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria
Email: *mn.ikegbunam@unizik.edu.ng

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Abstract

Nigeria is one of the malaria-endemic countries, where the treatment of malaria has relied heavily on natural and traditional medicines. This study was designed to investigate and ascertain the preference of *Eucalyptus camaldulensis* in treating malaria using three standard models among local herbalists. Extraction was carried out on the leaves of *Eucalyptus camadulensis* using methanol. The methanol crude extract and other solvent fractions obtained were used for analysis. An acute toxicity test (LD50) was carried out using Lorke's Method. The extract and its fractions were screened for phytochemical constituents using standard procedures. Different doses of the methanol crude extract (250 mg/kg, 500 mg/kg, and 1000 mg/kg) and other solvent fractions (250 mg/kg, 500 mg/kg) were assessed for their antiplasmodial property using the Suppressive, Curative, and Prophylactic models on different days. One hundred and eighty grams (18% w/w) of the extract were recovered from 1000 g of powdered leaves. The weight of fractions and their yields calculated from 50 g crude extract are n-hexane fraction (3.45 g, 6.9%), ethylacetate fraction (11.65 g, 23.3%), and butanol (7.84 g, 15.68%). The result of the acute toxicity test showed that the lethal dose of the plant was above 5000 mg/kg. For the crude extract, the 1000 mg/kg dose had the highest percentage of parasitemia suppression of 97.3%, 95.30%, and 75.97% in the curative, suppressive, and prophylactic models, respectively. The fractions exhibited a significant chemosuppressive effect when compared with the negative control, with the butanol fraction (500 mg/kg) showing a higher percentage suppression.

The findings in this study justify the use of this plant in traditional medicine for the management of malaria fever and tally with its folkloric use. However, more research is needed to establish the functions of the constituents in relation to antiplasmodial activity.

Keywords

Eucalyptus camadulensis, Antiplasmodial

1. Introduction

Malaria, a disease caused by the parasite *Plasmodium*, is a global health issue with over two million cases and over five hundred deaths reported each year, with a high percentage occurring in Sub-Saharan Africa [1]. *Plasmodium* species, namely *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae,* and *Plasmodium vivax*, are the main causal agents of malaria in humans. Also, a study by Muller *et al.* [2] has shown that two non-recombining species of *Ovale* exist (*Ovale curtisi*), which are not found in the same environment and thus do not encounter each other and *Ovale wallikeri* frequently. In Africa, *P. falciparum* is the most abundant *Plasmodium* species and accounts for about 98% of malaria deaths in Nigeria [1]. Nigeria alone accounts for a quarter of all malaria cases in Africa, recording about 30% - 50% morbidity and 25% mortality in infants [3]. *Anopheles gambiae* is the major carrier of malaria in Nigeria [3].

Malaria infection is commonly associated with the generation of Reactive Oxygen Species (ROS) by both the host and the parasite, which can lead to oxidative stress in the host system [4]. If left untreated, the prolonged infection can result in further systemic complications and even death [5] [6]. Despite being preventable and curable, malaria remains a pervasive tropical disease with a high incidence and mortality rate [7].

The WHO has set a goal to eradicate malaria entirely, but it continues to be a major health issue in Africa. The eradication plan faces challenges such as the continuous emergence and spread of drug-resistant parasites, Anopheles mosquitoes that are resistant to insecticides, the lack of an effective malaria vaccine [8], and an increase in the distribution of counterfeit antimalarial drugs [9]. In Nigeria, one of the countries where malaria is endemic, natural, and traditional medicines are frequently used for the treatment of malaria. Over 160 plant families and more than 1200 species of plants have been identified as traditional medicines for malaria treatment. Many of these plants have been scientifically verified for their efficacy against malaria through *in vitro* and/or *in vivo* tests [10].

The River Red Gum (*Eucalyptus camaldulensis* Dehn.) is a tree belonging to the *Eucalyptus* genus of the *Myrtaceae* family. This family includes 140 genera and about 3800 species distributed in tropical and subtropical regions worldwide [11]. *E. camaldulensis* is known to have diverse beneficial activities, including antiprotozoal effects [12]. Previous reports have highlighted its antiprotozoal ac-

tivity against *Trichomonas vaginalis*, a causative agent of trichomoniasis, the most prevalent nonviral sexual infection. Additionally, the antimalarial effect of *Eucalyptus camaldulensis* has been studied using curative tests with extraction solvents such as aqueous [13] and methanol [14] [15]. However, there are no studies investigating the antimalarial effect using prophylactic and 4-day suppressive tests that suggest its mechanism of action. Thus, this study was designed to investigate the preference of local herbalists in using *Eucalyptus camaldulensis* to treat malaria with different antimalarial models.

2. Materials and Methods

2.1. Plant Material Collection

The plant leaves were collected from Agulu Anambra State and were identified by the International Centre for Ethnomedicine and Drug Development with herbarium voucher number (InterCEDD/1689). The plant was air-dried at room temperature, pulverized into a coarse powder using a mechanical grinder, and stored in a container pending extraction

2.2. Experimental Animals

Experimental animals used for this study (Albino Wistar mice of both sexes weighing 18 - 29 g) were obtained from the veterinary medicine University of Nigeria Nsukka and housed in cages with sawdust as bedding at the animal house unit of the Department of Pharmacology and Toxicology. The animals were given a standard laboratory diet and water *ad libitum* and were allowed to acclimatize for one week prior to the experiment.

2.3. Preparation of the Extracts

The extraction was carried out by cold maceration; one kilogram of the plant was macerated in 2.5 l of methanol for 72 hrs after which the slurry was filtered through a muslin cloth and Whatman filter paper No1. The resulting filtrate was evaporated to dryness using a rotary evaporator and water bath (50 degrees Celsius). The extract was fractioned by liquid-liquid fractionation using butanol, ethyl acetate, and n-hexane in their increasing polarity. The extract was kept in an airtight container and stored at 4˚C for later use.

2.4. Phytochemical Analysis of the Extract

The crude plant extract was screened to identify various classes of chemical constituents following the methods described by Harborne (1973) [16]. The various tests carried out included tests for carbohydrates, reducing sugar, alkaloids, glycoside, saponins, tannins, flavonoids, proteins, oils, steroids and terpenoids, and acidic compounds

2.5. Acute Oral Toxicity Test

A test for acute oral toxicity was done on the extract and its fractions as described

by Lorke's method of determination of LD50 [17]. A total of 13 albino Wistar mice were used for this test.

At first, 9 of the animals were grouped into 3 groups, each with 3 participants. Each group was administered with an oral dose of 10 mg/kg, 100 mg/kg, and 1000 mg/kg of the extract respectively. After 24 hours, the animals were observed for death or behavioral changes. Going further, the four animals left were administered with doses of 2000 mg/kg, 3000 mg/kg, 4000 mg/kg, and 5000 mg/kg respectively after which the animals were observed for 24 hours for death or behavioral changes.

2.6. Normalization of Parasites in the Blood for Inoculation

The mouse infected by *P. berghei* and with different levels of parasitemia was used as donor mice. The donor mouse's parasitemia level was first determined from the blood that is obtained by cutting (0.5 to 1 mm section) the tail of the mice using scissors [17]. To inoculate and infect the study animals, the blood of the donor mouse with a parasitemia of 30% up to 37% was drained into a test tube containing anticoagulant (3.8% trisodium citrate (BDH Chemicals, England)) through the incision of the jugular vein. The collected blood was then diluted in normal saline to obtain 1×10^7 infected Red Blood Cells (RBCs) in every 0.2 ml suspension [18]. The dilution was done based on the erythrocyte count of the normal mice and parasitemia of the donor mice in such a way that 1 ml of blood contains 5×10^7 infected RBCs [16] [19]. Therefore, each mouse used was infected by 0.2 ml *P. berghei*-infected blood (1×10^7 parasitized RBCs) intraperitoneally.

2.7. 4-Day Suppressive Test

Evaluations of the antiplasmodial activity of the crude extract and its solvent fractions in early infection were carried out according to the procedure as described by Peter's 4-day suppressive test [20] [21]. Briefly, twenty-five mice were infected and randomly assigned into five groups with five mice for each. All groups were administered 0.2 ml of parasitized blood as described above for 3 hours post-infection. A daily oral administration was administered; 250 mg/kg, 500 mg/kg, 1000 mg/kg for the crude extract and 250 mg/kg, 500 mg/kg for the solvent fractions was administered from Day 1 to Day 4. On Day 4 post-infection, blood was collected from each mouse tail using clean, non-greasy, labelled frosted slides and smeared using a spreader to make thin films. Air-dried thin films were then fixed with a few drops of absolute methanol, left for approximately 10 - 15 minutes to air-dry, and stained for 15 minutes with 10% Giemsa stain at a pH of 7.2. The stain was washed off from the slides, and the slides were left to air-dry. The dried slides were then viewed through the light microscope using the oil immersion, and parasitemia was examined microscopically using the 100× objective. The parasitized RBCs were noted by the intracellular presence of the *Plasmodium* parasite. The parasitemia suppression percentage was calculated for each administered

dose by comparing the parasitemia densities in infected control mice with those of treated mice in six randomly selected fields of the microscope. 3% tween 80 was used as the negative control while 8 mg/kg of Lonart was a positive control. Each mouse's percentage parasitemia was determined on Day 4, and Day 7 post-infection while mice's body weight in g, rectal temperature in ˚C, and Packed Cell Volume (PCV) in % were reported just before the infection and on Day 4 post-infection.

2.8. Rane's (Curative) Test

Examination of the curative potential of the crude extract and its solvent fractions of the plant was done according to the methodology described by Peters [21] and Ryley [22]. The mice were intraperitoneally injected with standard inoculum on D0 (first day). After seventy-two hours, a daily oral administration was administered; 250 mg/kg, 500 mg/kg, 1000 mg/kg for the crude extract and 250 mg/kg, 500 mg/kg for the solvent fraction. The blood was collected from the tail of each mouse, and thin films were made on Day 4 and Day 7 post-infection to determine parasitemia levels. 3% tween 80 was used as the negative control while 8 mg/kg of Lonart was a positive control. The body weight of animals was measured using a weighing balance before and after treatment.

2.9. Prophylactic (Residual Infection) Test

The prophylactic activity of the crude extract and its solvent fractions was tested using the technique described by Peter's prophylactic test [21]. Twenty-five adult male mice were randomized into 5 groups with each group having 5 mice each and treated with the respective dose for 3 days; 250 mg/kg, 500 mg/kg, 1000 mg/kg (for the crude), 250 mg/kg, 500 mg/kg (for the solvent fraction), negative control (3% tween 80) and positive control (22.5 mg/kg of Sulphadoxine/pyrimethamine). Going forward, each animal was inoculated with 0.2 ml of the parasitized blood post-administration and followed up for 72 hours. The parasitemia level was recorded after 72 hours and 5 days post-infection. The body weight of animals in each group was measured using a weighing balance before and after treatment.

2.10. Statistical Analysis

The data obtained was analyzed using Graph Pad Prism (8.4.0.671). Results were presented as Mean ± Standard Error of Mean (SEM). Raw data were subjected to a one-way Analysis of Variance (ANOVA) followed by post hoc Bonferroni's multiple comparison test. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Percentage Extract Yields

The methanol leaf extract of Eucalyptus camaldulensis was dark green in color after concentration to dryness. One hundred and eighty grams (18% w/w) of the

extract was recovered from 1000 g of powdered leaves. The weight of fractions and their yields calculated from 50 g crude extract are n-hexane fraction (3.45 g, 6.9%), ethylacetate fraction (11.65 g, 23.3%), butanol (7.84 g, 15.68%).

3.2. Acute Toxicity

In the acute toxicity test in mice, the methanol leaf extract of *Eucalyptus camaldulensis* was administered orally up to a dose of 5000 mg, and no death was found in the mice after a 24 h observation period in the two phases of the tests. Also, there was generally an absence of symptoms of toxicity. The LD_{50} was therefore concluded to be greater than 5000 mg/kg.

3.3. Phytochemical Analysis of Extract and Fraction

Phytochemical test on methanol extract and ethyl acetate fraction gave a positive reaction for Alkaloids, flavonoids, Glycosides, Steroids, Saponins, Tannins, Terpenoids, Carbohydrates, Proteins, Oil, Acidic Compounds, and Reducing sugar. The n-hexane fraction showed a negative reaction for Alkaloids while the butanol fraction gave a negative reaction for steroids, terpenoids, and oil. The relative abundance of these components is shown in Table 1.

3.4. Curative Model

Effect of methanol crude extract of Eucalyptus camaldulensis on the established parasite (Days 4 and 7)

The extract at 250 mg/kg, 500 mg/kg, and 1000 mg/kg on Day 4 caused a

Table 1. The relative abundance of the phytochemicals present in the methanol crude.

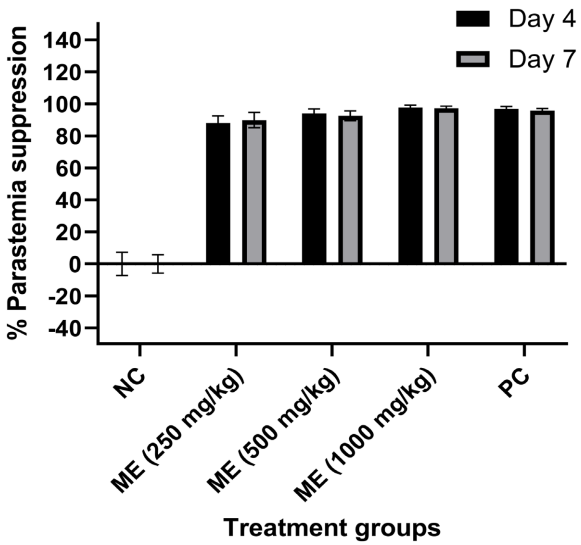
Secondary Metabolite	Relative Abundances			
	Methanol	n-Hexane	Ethyl Acetate	Butanol
Alkaloids	**	~	*	**
Flavonoids	***	***	*	**
Glycosides	**	**	*	**
Steroids	**	**	**	~
Saponins	***	*	**	***
Tannins	***	**	**	***
Terpenoids	**	*	*	~
Carbohydrate	**	**	**	**
Proteins	***	**	**	*
Oil	**	**	*	~
Acidic Compounds	*	*	**	*
Reducing Sugar	**	**	**	**

Key: ~Absent; *Present in Small Quantity; **Moderately Present; ***Abundantly Present.

dose-dependent increase in the parasitemia suppression level ($P < 0.05$) with a mean suppression of 88.15 %, 94.07%, and 97.7% respectively. However, the result of the parasitemia level on Day 7 post-infection showed a significant decrease in parasitemia with the mean percentage parasitemia suppression level of 89.86, 92.57, and 97.30 for the 250 mg/kg, 500 mg/kg, and 1000 mg/kg dose levels. Statistical analysis revealed that the extract at all dose levels significantly reduced the parasitemia level when compared to the negative control (Figure 1).

Effect of the Fractions on Established Parasite Days 4 and 7 Post-Infection

The infected mice were treated with 250 mg/kg and 500 mg/kg doses of the Butanol fraction, Ethyl acetate, and N-hexane fraction. The parasitemia count of the infected mice obtained on Day 4 revealed that the different doses of the fractions caused a significant increase in parasitemia suppression. The butanol fraction at 500 mg dose level was observed to be have a greater percentage mean value followed by the positive control, butanol fraction (250 mg/kg), n-hexane fraction 500 mg/kg, ethylacetate fraction 500 mg/kg, n-hexane fraction 250 mg/kg and ethylacetate fraction 250 mg/kg with a percentage suppression value of 100%, 96.99, 85.7%, 82.7%, and 78.9%. The groups treated with high doses of the fraction were observed to have suppressed the parasite more than the lower doses. The untreated group showed no suppression in parasitemia (Figure 2). The parasitemia of the treated and untreated group taken on Day 7 post-infection revealed that the doses of the fractions caused a significant increase in parasitemia suppression. The groups treated with the butanol fraction at dose levels of 250 mg/kg and 500 mg/kg showed a higher mean percentage suppression followed by groups treated with the PC (8 mg/kg), HF (500 mg/kg), EF (500 mg/kg), HF (250



NC = Negative Control; ME = Methanol Extract; PC = Positive Control. $P < 0.0001$ (compared to Negative Control).

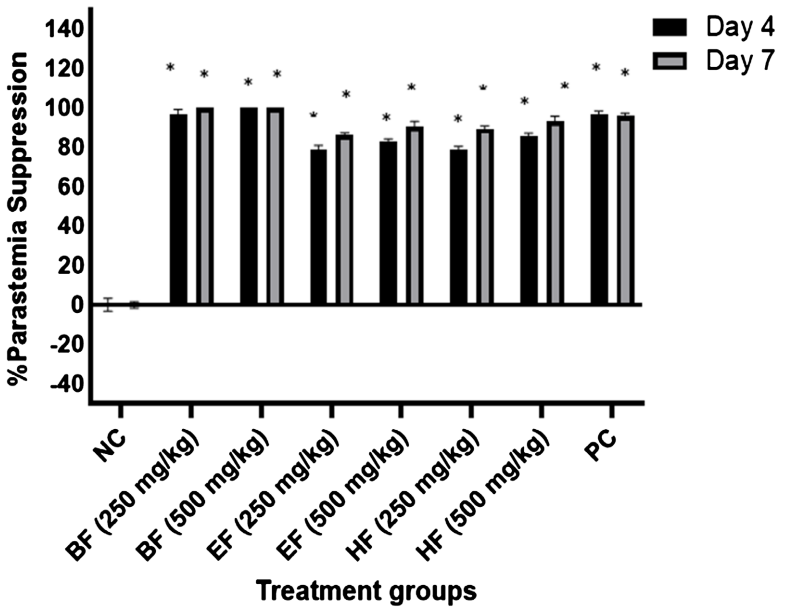
Figure 1. Curative effect of methanol crude extract of *Eucalyptus camadulensis* on parasitemia in *Plasmodium berghei* infected mice on Days 4 and 7 post-infection.

mg/kg) and EF (250 mg/kg) with a percentage suppression of 100%, 96%, 93.4%, 90.7%, 89.4%, and 86.1% respectively. The untreated group caused no suppression in parasitemia. As observed in the parasitemia count on Day 4 post-infection, the groups treated with the highest dose showed more increase in parasitemia suppression (Figure 2).

3.5. Suppressive Model

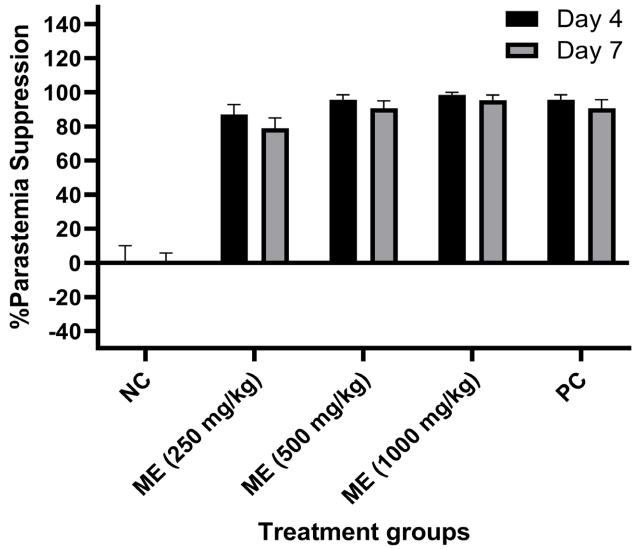
3.5.1. Suppressive Effect of the Methanol Extract on Parasitemia on Days 4 and 7 Post-Infection

The parasitemia level of the infected mice as determined on Day 4 post-infection using the suppressive model showed that the different doses of the extract caused a significant dose-dependent suppression in the parasitemia level as demonstrated in their mean percentage suppression levels of 86.96%, 95.65%, and 98.55% for the 250 mg/kg, 500 mg/kg and 100 mg/kg dose levels respectively. The untreated group/negative control showed no parasitemia suppression. The 500 mg/kg and 1000 mg/kg doses had a comparable and higher parasitemia suppression value when compared with the positive control respectively, though this was not statistically significant (Figure 3). The Parasitemia level of the infected mice as determined on Day 4 post-infection showed that the extract exhibited a significant dose-dependent decrease in parasitemia level with a percentage suppression value of 78.91%, 90.63%, and 95.31% for the 250 mg/kg, 500 mg/kg and 1000 mg/kg doses respectively. When compared with the negative control/untreated group, the doses of the extract demonstrated a statistically significant suppression ($P < 0.05$) (Figure 3).



NC = Negative Control; BF = Butanol Fraction; EF = Ethylacetate Fraction; HF = N-Hexane Fraction; PC = Positive Control; *P < 0.0001 (compared to Negative Control).

Figure 2. Curative Effect of *Eucalyptus camadulensis* solvent fractions on parasitemia in *Plasmodium berghei* infected mice on Days 4 and 7 post-infections.



NC = Negative Control; ME = Methanol Extract; PC = Positive Control; P < 0.0001 (compared to Negative Control).

Figure 3. Suppressive effect of methanol crude extract of *Eucalyptus camadulensis* on parasitemia in *Plasmodium berghei* infected mice on Days 4 and 7 post-infection.

3.5.2. Results Showing the Suppressive Effect of the Fractions on the Parasitemia Days 4 and 7 Post-Infection

The butanol, ethyl acetate, and n-hexane fractions were screened for their antiplasmodial effect using the suppressive model. The result of the Day 4 post-infection revealed that the fractions caused a significant increase in parasitemia suppression with a percentage value 91.3%, 81%, 89.6%, 87%, 98.2%, 95.6% and 97.4% for the groups treated with BF (250 mg/kg), BF (500 mg/kg), EF (250 mg/kg), EF (500 mg/kg), HF (250 mg/kg) HF (500 mg/kg) and the positive Control (PC) respectively. The groups treated with the Hexane fraction (250 mg/kg) caused a higher increase in parasitemia suppression which is comparable to the positive control. The untreated group caused no parasitemia suppression (**Figure 4**). On Day 7 post-infection, the different fractions of the extract significantly suppressed the parasite. The Hexane Fraction (250 mg/kg) caused a higher suppressive effect followed by the HF (500 mg/kg), Positive control, EF (500 mg/kg), EF (250 mg/kg), BF (250 mg/kg) and BF (500 mg/kg) with percentage suppressive value of 95.2%, 93.9%, 91.9%, 89.2%, 88.5%, 87.2% and 85.8% respectively. The untreated group recorded an increase in parasitemia (**Figure 4**).

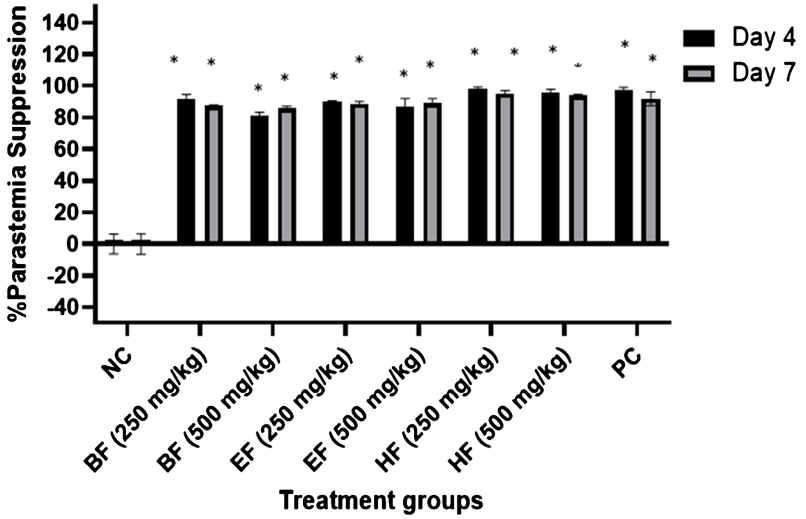
3.6. Repository Model

3.6.1. Prophylactic Effect of the Methanol Extract on Parasitemia Count Day 3 and Day 5 Post-Infection

After 72 hrs post-infection, the extract caused a dose-dependent change in parasitemia with a suppressive value of 71.76%, 75.29%, and 77.65% for the 250 mg/kg, 500 mg/kg and 1000 mg/kg respectively. The untreated/negative control group showed no suppression in parasitemia. The mean percentage suppressive

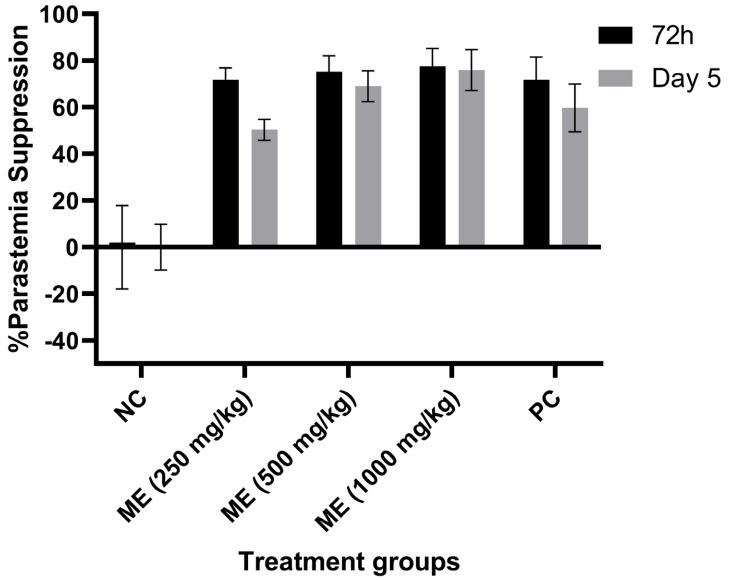
value of the positive control is comparable to the 250 mg/kg dose level of the extract (Figure 5).

On Day 5 post-infection, the value of the parasitemia count revealed that the different doses of the extract also caused a dose-dependent decrease in percentage parasitemia count with a percentage suppression value of 50.3, 68.9, and 75.9 for the 250 mg/kg, 500 mg/kg and 1000 mg/kg respectively. The negative control



NC = Negative Control; BF = Butanol Fraction; EF = Ethylacetate Fraction; HF = N-Hexane Fraction; PC = Positive Control; *P < 0.0001 (compared to Negative Control).

Figure 4. Suppressive effect of *Eucalyptus camadulensis* fractions on parasitemia in *Plasmodium berghei* infected mice on Days 4 and 7 post-infection.



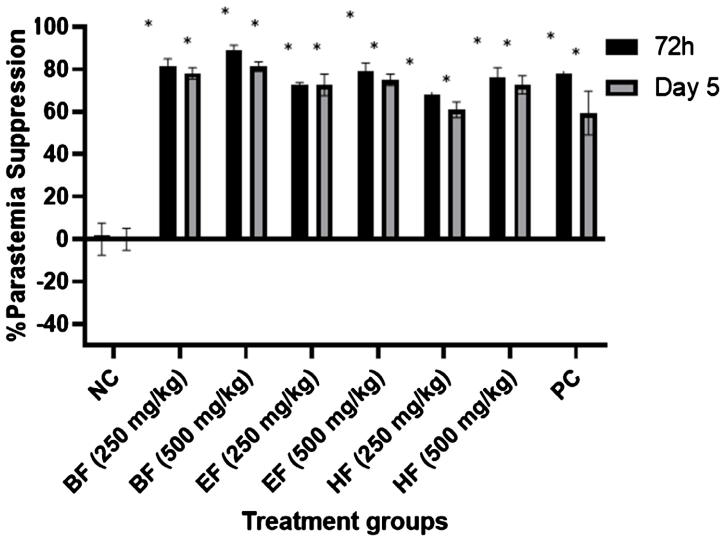
NC = Negative Control; ME = Methanol Extract; PC = Positive Control; P < 0.0001 (compared to Negative Control).

Figure 5. Prophylactic effect of methanol crude extract of *Eucalyptus camadulensis* on parasitemia in *Plasmodium berghei* infected mice on Day 72 hrs and Day 5 post-infection.

showed no suppression in parasitemia. Statistical analysis showed that there was a significant change in parasitemia count when compared to the negative/untreated (Figure 5).

3.6.2. Prophylactic Effect of the Fractions on Parasitemia Day 3 and Day 5 Post-Infection

The fractions demonstrated a significant and dose-dependent decrease in parasitemia count at the experimental dose of 250 and 500 mg/kg used. The mean percentage suppression produced by BF, EF, and HF were (81.6%, 88.9%), (72.4%, 78.9%) and (67.8%, 76.1%) respectively while the group treated with sulphadoxine-pyrimethamine at 22.5 mg/kg produced a mean percentage parasitemia suppression of 77.9% (Figure 6). On Day 5 post-infection, the fractions demonstrated a significant and dose-dependent decrease in parasite count at test doses of 250 and 500 mg/kg used. The BF, EF, and HF produced a mean percentage suppression of 78.1%, 81.2%, 72.6%, 75%, 60.9%, and 72.6% respectively while the group treated with the positive control caused a mean percentage suppression of 59.38. The untreated group produced no parasite suppression in the infected mice (Figure 6).



NC = Negative Control; BF = Butanol Fraction; EF = Ethylacetate Fraction; HF = N-Hexane Fraction; PC = Positive Control; *P < 0.0001 (compared to Negative Control).

Figure 6. Prophylactic effect of *Eucalyptus camadulensis* fractions on parasitemia in *Plasmodium berghei* infected mice on Day 72 hrs and Day 5 post-infection.

4. Discussion

In Nigeria and Africa, malaria is a significant economic burden due to the increasing resistance of *Plasmodium falciparum* to conventional antimalarial drugs. Therefore, new and more efficient antimalarial drugs are needed to achieve the new global target of zero-malaria status by 2030. This study investigated the *in vivo* antimalarial effect of methanol extract and different solvent fractions of *Eucalyptus camadulensis* (Denhn) using the parasite *Plasmodium berghei*. The

study also used drugs that are sensitive to the parasite and have been used in humans as standards. The LD50 of the crude extract was >5000 mg/kg, indicating that the extract is safe for use in the treatment of malaria and other diseases [22].

This study evaluated the antiplasmodial effect of the methanol crude extract using suppressive, curative, and prophylactic models. The suppressive test showed that all doses of the plant had an antimalarial effect when compared to the negative control on different days. Using the curative model, all doses of the extract revealed a significant chemo-suppressive effect on Days 4 and 7 when compared to the negative control, confirming its antimalarial effect. The prophylactic effect of the methanol extract revealed that all doses of the plant had a significant chemosuppressive effect, with the highest dose showing a percentage suppression of 77.65% and 75.97% at 72 hrs and Day 5 post-infection. However, the chemoprophylactic effect was lower than the 4-day suppressive and curative test, which could be due to rapid clearance or metabolism of the active component responsible for the antiplasmodial effect. Also, there might be a possibility that the extract acted through metabolic activation of the immune system, and hence the parasite clearance could not be total [23]. This finding agrees with other studies in which the percentage of parasitemia in the suppressive and curative tests was lower than in the prophylactic test [24]. In general, the models showed a dose-dependent decrease in parasitemia levels as observed in the reports of Unekwuojo *et al.* [25], Muluye *et al.* [26], Habte *et al.* [27], and Orabueze *et al.* [28].

The antiplasmodial effect of the methanol crude extract observed in this study could be linked to the synergism of the individual phytochemicals. The phytochemical analysis of the crude extract revealed secondary metabolites such as tannins, saponins, flavonoids, and terpenoids that have been implicated in antimalarial activities. These metabolites disrupt the parasite's ability to detoxify heme into a non-toxic pigment, block parasite synthesis, chelate with nucleic acids, and display free radical scavenging effects [28].

The fractions exhibited a significant chemosuppressive effect, with the butanol fraction (500 mg/kg) showing a higher percentage suppression. The chemoprophylactic effect was lower compared to the curative and suppressive models, and the butanol fraction appeared to have a better antiplasmodial effect than the other fractions. Overall, the results indicate that *Eucalyptus camadulensis* has significant antimalarial activity and could be a potential source of new antimalarial drugs.

In conclusion, our study demonstrated that the crude methanol extract and the butanol fraction of *Eucalyptus camadulensis* possess significant antiplasmodial activity in the three models used. The antiplasmodial effect was dose-dependent, and the crude extract and the butanol fraction showed a greater effect in reducing the level of parasitemia in circulation. These findings suggest that *Eucalyptus camadulensis* could serve as a new source for the development of plant-based antimalarial agents. Furthermore, our study validates the traditional use of this plant

for the management of malaria fever. However, further research is required to determine the mechanism of action of the plant's constituents in relation to its antiplasmodial activity.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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