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In vivo anti-malarial activities of methanol and aqueous extract of stem bark of Eucalyptus camaldulensis on Plasmodium berghei-berghei

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Abstract

Malaria is a life-threatening infectious disease transmitted by mosquitoes, affecting both humans and animals. It is a blood-borne illness caused by the Plasmodium parasite. This research was aimed at probing the in vivo anti-malarial effects of aqueous and methanol extracts of Eucalyptus camaldulensis stem bark. Two hundred (200) grams of the pulverized plant material were sequentially macerated in 900 mL of methanol and water for 24 hours, respectively. In each case, the mixture was filtered using the Whatman filter paper. The filtrate was then evaporated to dryness in a water bath to obtain the crude extract. Twenty-seven (27) albino mice of the same sex, weighing 18-25 g, were selectively grouped into five groups of three mice each. Groups 1, 2, and 3 were infected but treated with the aqueous and methanol extracts of Eucalyptus camaldulensis stem bark at doses of 50, 100, and 150 mg/kg body weight for five days. Group 4 animals were infected and treated with 25 mg/kg per body weight of chloroquine, while group 5 was left untreated and served as a negative control group. It was detected that the animals administered with both extracts showed a significant ($p<0.05$) decrease in parasitemia when compared with the negative control group. However, the group treated with chloroquine at 25 mg/kg body weight showed a significantly lower parasitemia ($p<0.05$) when compared with the groups treated with both methanol and aqueous extract. The dose-by-dose comparison of the anti-malarial effect of the two extracts at the dose of 150 mg/kg body weight indicates that the methanol extract exhibited higher anti-malarial activity when compared with the aqueous extract at the same dose. The results of this study indicate that the methanol and aqueous extracts of the stem bark of Eucalyptus camaldulensis exhibit anti-malarial activity, supporting its traditional medicinal use.

Keywords: Antimalarial, *Eucalyptus camaldulensis*, Parasitemia, *Plasmodium berghei-berghei*

1. Introduction

For centuries, medicinal plants have been used globally to treat various ailments, including stomach pain, diabetes, hyperacidity, gonorrhea, dysentery, cystitis, urethritis, laryngitis, leucorrhoea,

inflammation, bronchitis, tuberculosis, and wounds. It is estimated that approximately 80% of the global population, especially in developing countries, rely on traditional medicine, including herbal formulations, for their primary health care needs (World Health Organization, 2023). The demand for medicinal plants has significantly increased in countries such as China, South Africa, and India, prompting extensive research into their sustainable use and conservation (Chan *et al*., 2016). Traditional medicine has played a crucial role in treating malaria and other illnesses for generations. This has led to the development of two core classes of modern antimalarial drugs: quinoline derivatives (e.g., chloroquine, mefloquine, piperaquine) and artemisinin-based medications, including both natural and semisynthetic derivatives (World Health Organization, 2022; Makanga & Premji, 2024).

Several medicinal plants have been identified as effective in malaria treatment, including *Canna indica, Aloe vera, Adansonia digitata, Mangifera indica, Enantia chlorantha, Carica papaya, Psidium guajava*, and *Artemisia annua* from which artemisinin was derived.

Eucalyptus camaldulensis, commonly known as the "red river gum" and referred to as "Itchen Turare" in Hausa, is a species of the *Eucalyptus* genus. This large evergreen tree, reaching heights of 20–40 meters, is distinguished by its stout trunk and irregularly shedding bark, which reveals white, yellow, and grey patches while becoming rough at the base (Dickson, 2011). Although native to Australia, the species is widely distributed across Nigeria and other regions. The medicinal properties of *Eucalyptus camaldulensis* have been widely recognized. Its essential oil is widely used in treating colds and coughs, while a gum from the plant, when boiled with water and sugar, is traditionally consumed to soothe lung problems and toothaches. Additionally, a bark infusion has been employed in folk medicine to treat eye infections and diarrhea (Wikipedia, 2025). This plant is a rich source of bioactive compounds, including steroids, alkaloids, tannins, saponins, terpenes, flavonoids, polyphenolics, phenolics, triterpenoids, fatty acids, lignin, vitamin C, anthraquinones, glycosides, anthocyanins, coumarins, cardiac glycosides, and volatile oils (Ghalem et al., 2014). Several studies have reported that the oils and secondary metabolites of various *Eucalyptus* species possess antimicrobial and antifungal properties. Many species within this genus are also used in traditional medicine for treating malaria, microbial infections, and dysentery (Beenken, 2017).

2. Literature review

Malaria is a life-threatening disease caused by **Plasmodium** parasites and transmitted through the bite of infected female *Anopheles* mosquitoes (WHO, 2019). The parasite enters the human bloodstream through mosquito saliva, migrates to the liver, and multiplies before infecting red blood cells (Caraballo, 2014). There are five known *Plasmodium* species responsible for malaria, including *P. falciparum, P. malariae, P. ovale, P. vivax, and P. knowlesi.* In Nigeria, *Plasmodium falciparum* and *P. malariae* are the predominant malaria-causing parasites. The economic toll malaria takes on Nigerian communities is staggering—households in endemic areas spend up to **7% of their monthly income** on malaria treatment, and the disease costs the national economy more than US$1.1 billion per year, primarily due to lost productivity and rising out-of-pocket healthcare expenses (Punch Nigeria 2024). A major challenge in malaria control is the growing resistance of *Plasmodium* species—particularly *P. falciparum*—to commonly used antimalarial drugs such as chloroquine, sulfadoxine–pyrimethamine, and even artemisinin-based combination therapies (ACTs) (Conrad & Rosenthal,

2019). The reduced efficacy of chloroquine prompted significant research efforts, culminating in the development of artemisinin-based combination therapies (ACTs), which remain the cornerstone of malaria treatment (World Health Organization, 2024).

Despite the proven success of ACTs, many malaria-endemic communities continue to rely heavily on traditional herbal remedies due to their accessibility, familiarity, and affordability (World Health Organization, 2024). Malaria remains widespread in regions characterized by high temperatures, humidity, and stagnant water—ideal breeding grounds for *Anopheles* mosquitoes. Subtropical and tropical regions such as Asia, Latin America, Eastern Europe, and Africa report the highest infection rates. Sub-Saharan Africa, in particular, bears an overwhelming burden, accounting for approximately 94% of malaria cases and 95% of malaria-related deaths in 2023 (World Health Organization, 2024; Reuters, 2024). Common symptoms include fever, chills, headaches, muscle aches, vomiting, shivering, jaundice, hemolytic anemia, retinal damage, hemoglobinuria, and seizures. These typically emerge 10–15 days post-infection (World Health Organization, 2024). Moreover, even individuals taking preventive antimalarial drugs may still exhibit symptoms in some cases (World Health Organization, 2024). Given the medicinal significance of *Eucalyptus camaldulensis*, this study aims to evaluate the in vivo anti-malarial activities of its methanol and aqueous stem bark extracts against *Plasmodium berghei-berghei* in mice.

3. Research methodology

3.1. Materials and methods

Collection and preparation of the sample

The stem bark of *Eucalyptus camaldulensis* was harvested from the IBB Guest House in Minna, Niger State, Nigeria. It was then air-dried at room temperature and ground into a fine powder using a mortar and pestle.

Approval for Ethical clearance.

Approval for this research was requested and granted by the Animal Ethics and Research Committee of IBBU, Lapai, Niger state of Nigeria, prior to initiating the experimental study.

Animals used for the Experiment

Albino mice of the same sex, weighing between 18 and 25 g, were procured from the Nigerian Institute of Trypanosomiasis Research (NITR), Kaduna, Kaduna State, Nigeria. The animals were kept under standard conditions with access to feed and water.

The Plasmodium Bergei Parasite

The malaria parasite (*Plasmodium berghei*, Anka strain) used for transfection was sourced from the National Institute for Medical Research (NIMR), Lagos, Nigeria. It was administered through intraperitoneal injection of infected blood from one mouse to another.

Plant Extracts Preparation

The extract was prepared following the method outlined by Musa et al. (2013). A total of 200 g of the pulverized *Eucalyptus camaldulensis* stem bark was subjected to cold maceration in 900 mL of

methanol for 24 hours. The mixture was then filtered using Whatman No. 1 filter paper, and the remaining residue was further macerated in 900 mL of distilled water under the same conditions. After another 24 hours, the mixture was filtered again using Whatman No. 1 filter paper. The collected filtrate was concentrated to dryness using a water bath, and the yield of the crude extract was stored for further analysis

Determination of Extract Yield

The extract yield was calculated using the following formula:

$$\text{Yield of Percent (\%) extract} = \frac{\text{Extract Weight}}{\text{Sample Weight}} \times 100$$

Experimental Animals Acclimatization

The animals were acclimatized for a period of four-week in their new environment. During the period, the animals were feed with their standard diet and water.

Animals Grouping

The infected mice were divided into five groups, with each group consisting of three animals. For identification purposes, the animals were marked on the head, trunk, leg, arm, and tail.

In vivo Antimalarial Activity Assay

The experimental procedure was adapted from the classical Peters (1965) 4-day suppressive test of *Plasmodium berghei* infection in rodents, with slight modifications following Johnson and Momoh (Johnson & Momoh, 2015). Briefly, each groups of mice were intraperitoneally inoculated with 0.2 mL of infected red blood cells containing approximately 1×10^7 parasites. Twenty-four hours post-infection, treatment began and continued daily for five days using methanol and aqueous plant extracts at doses of 50, 100, and 150 mg/kg body weight. A positive control group received chloroquine at 25 mg/kg body weight, while a negative control group received the solvent only.

Albino mice infected with *Plasmodium berghei* served as donor mice. The donor mouse was euthanized using chloroform anesthesia, and blood was collected directly from the heart via cardiac puncture into an EDTA bottle. The collected blood was then diluted with normal saline, adjusted according to the parasitemia level and red blood cell count of the donor mice.

Extracts Administration

The extracts were administered to the infected mice 24 hours after infection, following confirmation of parasitemia levels. Groups 1 to 3 received 50 mg/kg, 100 mg/kg, and 150 mg/kg body weight of the plant extracts (methanol and aqueous extracts of *Eucalyptus camaldulensis* stem bark), respectively. Mice in Group 4 were treated with chloroquine at a dose of 25 mg/kg body weight, while those in Group 5 remained untreated as the negative control.

Experimental Animals Monitoring, the Parasitemia Level in Experimental Animals

Parasitemia was assessed 24 hours after treatment and subsequently on days 5 and 7 through microscopic examination. This involved counting parasites in four different fields, with approximately 100 red blood cells per field, from thin blood films. Blood samples were collected from

the experimental mice by carefully cutting the tip of the tail, placing a drop of blood on a clean glass slide, and smearing it evenly. The smear was then fixed with methanol and stained using a 10% Giemsa solution. The prepared slides were examined under a microscope at 100× magnification, and parasitemia levels were determined for all animals. The percentage parasitemia (% parasitemia) was calculated using the following formula:

$$\% \text{ Parasitemia} = \frac{\text{No. of RBC parasitized}}{\text{No. of RBC Total}} \times 100$$

Tests for Acute Toxicity

The acute oral toxicity of the methanol and aqueous extracts of *Eucalyptus camaldulensis* stem bark was assessed following the method outlined by Abdulmumin et al (Abdulmumin et al., 2021). The animals were divided into four groups, each consisting of four mice. Each group received an oral dose of 300 mg/kg, 500 mg/kg, 2000 mg/kg, and 3000 mg/kg body weight, respectively. Signs of toxicity, including mortality, were observed and recorded within 24 hours.

Statistical Analysis

The results were presented as mean ± standard deviation and analyzed using the Statistical Package for Social Sciences (SPSS) software. A one-way analysis of variance (ANOVA) was conducted to compare group means, with a significance level (α) set at 0.05. A p-value of less than 0.05 was considered statistically significant.

4. Result

Extracts Percentage Yields.

Table 1: Percentage yield of the extract

Solvents	Percentage yield (%)
Cyclohexane	0.11
Dichloromethane	0.40
Methanol	0.95
Aqueous	2.1

From the table above, the aqueous extract (2.1) shows the highest percentage yield, followed by the methanol extract (0.95) and cyclohexane extract with least yield.

In Vivo Anti-Malarial Activity of Methanol Extract of Stem Bark of *Eucalyptus Camaldulensis*

Table 2: presents the parasitemia levels in infected mice following treatment with the methanol extract of *Eucalyptus camaldulensis* stem bark.

Concentration (mg/kg body weight)	Parasitemia (mean ± 1 SD)		
	Day 1	Day 5	Day 7
150	11.13 ± 0.52^{c}	4.25 ± 0.64^{c}	2.13 ± 0.92^{b}
100	11.23 ± 0.52^{b}	6.88 ± 0.52^{b}	5.63 ± 0.52^{b}

50	**11.75 ± 0.35^{b}**	**8.50 ± 0.52^{a}**	**2.13 ± 0.54^{a}**
25 (chloroquine)	**12.50 ± 0.71^{a}**	**3.87 ± 0.76^{d}**	**1.13 ± 0.52^{c}**
0 (control)	**12.85 ± 0.54^{d}**	**19.50 ± 0.52^{e}**	**27.23 ± 0.56^{c}**

Values with the same superscripts have no significant difference (p >0.05)

The *in vivo* anti-malarial efficacy of the methanol and aqueous stem bark extracts of *Eucalyptus camaldulensis* was assessed in albino mice. Three dosage levels were administered: 150 mg/kg, 100 mg/kg, and 50 mg/kg body weight. Additionally, the effects of a standard anti-malarial drug, chloroquine (25 mg/kg), were evaluated and presented in Table 2.

Table 2 illustrates the percentage parasitemia in mice inoculated with *Plasmodium berghei berghei*. A statistically significant difference ($p < 0.05$) was observed in parasitemia levels between the treated and untreated groups on day 5 post-inoculation, with treated mice exhibiting markedly lower parasitemia compared to the negative control group. A further significant reduction in parasitemia was noted on day 7 post-infection. The percentage parasitemia in extract-treated groups ranged from 4.25% to 6.88%. Comparing parasitemia levels on day 7 with those on day 5 revealed a steady decline, achieving suppression rates of 60% to 70%. Among the extract-treated groups, the methanol extract at 150 mg/kg body weight resulted in the lowest parasitemia levels compared to the other dose groups (Table 2). However, the group treated with the standard drug, chloroquine, exhibited a significantly lower parasitemia level than the extract-treated groups. Among the three extract doses, the 150 mg/kg methanol extract demonstrated the greatest reduction in parasitemia, suggesting a dose-dependent response.

Anti-Malaria Property of Aqueous Extract of Stem Bark Of *Eucalyptus Camaldulensis*

Table 3: *In Vivo* Aqueous Extract Anti-Malaria Property of the Stem Bark of *Eucalyptus Camaldulensis.*

Concentration (mg/kg body weight)	**Parasitemia (mean ± 1 SD)**		
	Day 1	**Day 5**	**Day 7**
150.00	**10.50 ± 0.52^{c}**	**6.13 ± 0.64^{c}**	**3.63 ± 0.92^{b}**
100.00	**10.75 ± 0.52^{b}**	**8.75 ± 0.52^{b}**	**7.63 ± 0.52^{b}**
50.00	**11.75 ± 0.35^{b}**	**9.75 ± 0.52^{a}**	**7.83 ± 0.54^{a}**
25.00 (chloroquine)	**10.38 ± 0.71^{a}**	**3.13 ± 0.76^{d}**	**1.13 ± 0.52^{c}**
0.00 (control)	**11.38 ± 0.54^{d}**	**17.50 ± 0.52^{e}**	**23.13 ± 0.54^{c}**

Values in each column with the same superscripts have no significant difference (p> 0.05)

Table 3 presents the parasitemia levels in infected mice treated with the aqueous extract of *Eucalyptus camaldulensis* stem bark. Values within each column that share the same superscripts indicate no significant difference ($p > 0.05$).

Table 3 presents the percentage parasitemia in mice infected with *Plasmodium berghei.* A statistically significant difference ($p < 0.05$) was observed between the treated and untreated groups, with the chloroquine-treated group exhibiting a markedly lower parasitemia level compared to the group receiving the highest extract dose (150 mg/kg body weight). By day 5 post-inoculation, the

untreated group displayed significantly higher parasitemia levels than all treated groups; including the reference drug group (Table 3). Additionally, the standard drug-treated group showed a significantly lower parasitemia level compared to those treated with the aqueous extract. Among the extract-treated groups, mice receiving the 150 mg/kg dose had the lowest percentage parasitemia compared to the untreated group and lower dosage groups. Values within each column that share the same superscripts indicate no statistically significant difference ($p > 0.05$). At a dosage of 150 mg/kg, the methanol extract of *Eucalyptus camaldulensis* stem bark exhibited a significantly greater reduction in parasitemia levels compared to the aqueous extract.

Table 4: Comparative anti-malarial efficacy of 150 mg/kg methanol and aqueous extracts of *Eucalyptus camaldulensis* stem bark and chloroquine (25 mg/kg).

Concentration (mg/kg body weight)	Parasitemia (mean ± 1 SD)		
	Day 1	Day 5	Day 7
Methanol	11.13 ± 0.76^{c}	4.25 ± 0.54^{a}	2.13 ± 0.76^{a}
Aqueous	10.75 ± 0.54^{b}	6.13 ± 0.52^{b}	3.63 ± 0.46^{b}
Chloroquine (25 mg/kg)	12.25 ± 0.71^{b}	3.87 ± 0.77^{b}	1.13 0.52^{b}

Values in each column with the same superscripts have no significant difference ($p > 0.05$)

At 150 mg/kg, the methanol extract of the stem bark of *Eucalyptus camadulensis* significantly reduced the level of parasitemia compared to the aqueous.

Table 5: Comparative Anti-Malarial Effect of 100 mg/kg Methanol and Aqueous Extracts of *Eucalyptus camaldulensis* Stem Bark and Chloroquine (25 mg/kg).

Concentration (mg/kg body weight)	Parasitemia (mean ± 1 SD)		
	Day 1	Day 5	Day 7
Methanol	11.00 ± 0.52^{a}	6.88 ± 0.71^{a}	5.63 ± 0.71^{a}
Aqueous	10.75 ± 0.52^{a}	8.85 ± 0.99^{a}	7.63 ± 0.52^{a}
Chloroquine (25 mg/kg)	12.00 ± 0.79^{c}	3.87 ± 0.77^{c}	1.13 ± 0.52^{c}

Values in each column with identical superscripts indicate no significant difference ($p > 0.05$).

The methanol extract of *Eucalyptus camaldulensis* stem bark at a dosage of 100 mg/kg demonstrated notable anti-malarial activity compared to the aqueous extract on both day five and day seven. Additionally, mice treated with 25 mg/kg of chloroquine exhibited a lower number of infected red blood cells.

Table 6: Comparative Anti-Malarial Effect of 50 mg/kg Methanol and Aqueous Extract of the Stem Bark of *Eucalyptus camaldulensis* and Chloroquine (25 mg/kg).

Concentration (mg/kg body weight)	Parasitemia (mean ± 1 SD)		
	Day 1	Day 5	Day 7
Methanol	11.75 ± 0.84^{ab}	8.50 ± 0.74^{a}	7.63 ± 1.71^{a}
Aqueous	11.75 ± 0.52^{a}	9.75 ± 0.71^{b}	7.83 ± 0.54^{b}
Chloroquine (25 mg/kg)	12.25 ± 0.71^{b}	3.87 ± 0.77^{c}	1.13 ± 0.52^{b}

Values in each column with the same superscripts have no significant difference ($p > 0.05$)

At a dose of 50 mg/kg body weight, both the methanol and aqueous extracts exhibited a significantly higher level of parasitemia on days five and seven compared to the reference drug, chloroquine, administered at 25 mg/kg body weight on the same days.

Acute Toxicity Assessment of *Eucalyptus camaldulensis* Stem Bark

The acute toxicity evaluation of the extracts was conducted following the method outlined by the British Toxicology Society. Twenty-four hours after administering the extracts, no mortality was observed, indicating that the extracts were well tolerated by the mice even at the highest dose of 3000 mg/kg. To further assess potential toxicity, internal organs, including the kidneys, heart, spleen, liver, and lungs, were examined post-dissection. No visible signs of toxicity were detected in any of the organs. Therefore, it can be concluded that the methanol and aqueous extracts of *Eucalyptus camaldulensis* exhibit no apparent physical or internal toxic effects on mice at the evaluated dosage levels.

5. Discussion

The acute toxicity study of the methanol and aqueous extracts of *Eucalyptus camaldulensis* stem bark was assessed in albino mice. The administered doses were 300, 500, 2000, and 3000 mg/kg body weight, respectively. After 24 hours of observation, no mortality was recorded, indicating that the extracts were well tolerated. The findings of this study revealed that the aqueous extract of *Eucalyptus camaldulensis* stem bark exhibited significant ($p < 0.05$) anti-malarial activity. Mice treated with the extract showed a significantly ($p < 0.05$) lower parasitemia level compared to the untreated control group. However, the anti-malarial activity of the aqueous extract differed significantly ($p > 0.05$) from that of the standard drug, chloroquine (25 mg/kg body weight), on days 5 and 7 post-infection. Similarly, the methanol extract of *Eucalyptus camaldulensis* stem bark also demonstrated significant ($p < 0.05$) anti-malarial activity. The three groups of mice treated with the methanol extract had significantly ($p < 0.05$) lower parasitemia levels than the negative control group. When compared to the chloroquine-treated group, mice treated with the methanol extract initially exhibited lower parasitemia levels on day 1 post-infection; however, by days 5 and 7, the parasitemia levels in the methanol extract-treated groups were higher than those observed in the reference drug group.

The observed anti-malarial effects of both extracts may be attributed to the presence of bioactive compounds. According to Sani et al. (2014), the medicinal properties of plants are due to the presence of phytochemicals. A comparative evaluation of the dose-dependent anti-malarial effects of both extracts (Tables 4 to 6) indicated that the methanol extract exhibited a significantly higher anti-malarial activity than the aqueous extract. This variation in activity could be attributed to differences in the types and concentrations of secondary metabolites extracted by each solvent. Previous studies have shown that different solvents extract varying phytochemicals from the same plant material. Ibrahim *et al*. (2016) reported that terpenoids, steroids, glycosides, tannins, saponins, phenols, and anthraquinones were present in both the methanol and aqueous stem bark extracts of *Eucalyptus camaldulensis*. However, alkaloids were found only in the methanol extract, whereas glycosides and anthraquinones were detected in the aqueous extract but were absent in the methanol extract.

6. Conclusions

The findings of this study demonstrate that both the aqueous and methanol extracts of *Eucalyptus camaldulensis* stem bark possess anti-malarial properties, supporting its traditional medicinal use. Further efforts should be directed towards purifying the crude extracts to isolate and identify the specific bioactive compounds responsible for the observed effects. Additionally, future studies could explore the anti-malarial potential of other parts of *Eucalyptus camaldulensis*, such as its seeds or roots, against *Plasmodium berghei berghei*. Efforts should be made to improve upon *Eucalyptus camaldulensis* by purifying the crude extract in order to identify and isolate the bioactive principle. However, further research may be conducted using the seed or the root of *Eucalyptus camaldulensis* against *plasmodium berghei-berghei.t*

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