BAGHDAD JOURNAL OF BIOCHEMISTRY AND APPLIED BIOLOGICAL SCIENCES
2024, VOL. 5, NO. 1, 59-71, e-ISSN: 2706-9915, p-ISSN: 2706-9907
https://doi.org/10.47419/bjbabs.v5i01.269

ORIGINAL ARTICLE

Methanol Extract of Ficus trigonata Stem Bark Demonstrated Antiplasmodial Activity in Mice

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ABSTRACT

Malaria, caused by Plasmodium parasites, remains a global health concern, with drug resistance complicating control efforts. Plant-derived compounds have emerged as potential antimalarial agents. This study explores the effectiveness of methanol extract of Ficus trigonata stem bark against Plasmodium berghei in a murine model. Thirty (30) adult mice used in this study were divided into 5 groups of 6 mice per group. Group A was inoculated with P. berghei and not treated which served as negative control. Group B was inoculated and treated with 10mg/Kg body weight of chloroquine (standard control). Groups C, D and E were inoculated with Plasmodium berghei and treated with the extract in doses of 100, 200 and 300 mg/kg body weight respectively. Parameters such as Packed cell volume (PCV) was measured by hematocrit technique, Parasitemia levels were monitored by microscopy, and Chemo-suppression activity was calculated to assess antiplasmodial potential. The in vivo murine model that was used enables efficient preliminary screening with good predictive validity. Ficus trigonata stem bark extract showed dose-dependent antiplasmodial activity in Plasmodium berghei-infected mice. All doses (100-300mg/kg) significantly ($p<0.05$) increased the level of packed cell volume on days 1-3 post-treatment. Parasitemia levels reduced significantly ($p<0.05$), with 300 mg/kg approaching chloroquine efficacy. Impressive dose-dependent chemo-suppression reached 85.68% with the highest F. trigonata stem bark extract dose by day 4, nearing the 94.16% standard drug activity. These findings highlight the extract's potential as an antimalarial agent and contribute to the search for alternative treatments.

Keywords Ficus trigonata, Malaria, Packed Cell Volume, Parasitemia and Plasmodium berghei, Chemo-suppression

Received 2023-11-24
Revised 2024-01-13
Accepted 2024-03-1

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DOI https://doi.org/10.47419/bjbabs.v5i01.269

Pages: 59-71

INTRODUCTION

The etiological agent responsible for malaria is the Plasmodium parasite, an organism transmitted through the bites of mosquitoes. This mosquito-borne parasitic illness contin-

ues to pose a substantial threat to global health, particularly in regions where the infection is endemic. Malaria continues to be a major global source of morbidity and mortality despite continuous attempts to battle this debilitating illness, with pregnant women and children being the most vulnerable groups.[1] The development of Plasmodium strains that are resistant to drugs has made it more difficult to manage and eradicate the illness.[2]

Research throughout the years has looked at a number of ways to find novel antimalarial drugs, including naturally occurring plant-based compounds, which have been crucial in the development of effective medicinal agents.[3] These organic compounds frequently act as inspiration, presenting fresh approaches combating malaria.[4]

Ficus trigonata, possessing an extensive historical legacy of traditional application across diverse geographical regions, emerges as a noteworthy natural product currently undergoing rigorous scientific scrutiny.[5] Ficus species have garnered significant interest in their prospective medicinal use, mainly because of their varied phytochemical composition and proven antiparasitic properties.[6] Extracts from Ficus carica showed effectiveness against Leishmania major in both in vitro and in vivo models, reducing lesion size and parasite burden.[7] A study also found that a mixture of Ficus and olive leaf extracts reduced oocyst shedding and improved intestinal histopathological changes in immunocompromised mice infected with Cryptosporidium parvum.[8]

Within this framework, our study aims to investigate the effectiveness of Ficus trigonata's methanol stem bark extract against Plasmodium berghei, a rodent parasite that is frequently used as a model for in vivo malaria research. In particular, we investigate the effect of the extract on important malaria infection indicators such as PCV (packed cell volume), parasitemia, and chemo-suppressive action.

Chemo-suppression refers to the capability of an agent to inhibit malaria parasite growth and multiplication within host red blood cells, thereby reducing overall parasitemia.[9] Assessing chemo-suppressive potential provides crucial information about efficacy in controlling parasite proliferation and preventing severe manifestations. Many standard antimalarials like chloroquine exert effects via chemo-suppression of erythrocytic stage Plasmodium.[10] Given rising resistance, identifying agents with chemo-suppressive effects even on resistant parasites is especially valuable.

These parameters provide information on both the disease's severity and the efficacy of possible treatments. The decision to examine these factors stems from the paramount clinical significance in the context of malaria. A decrease in PCV, which is a symptom of malaria caused by the parasite's destruction of red blood cells, is a crucial sign in the treatment of the illness.[11] PCV represents the percentage of red blood cells in the circulation. In a similar vein, parasitemia levels and Plasmodium infection severity are closely correlated, with lower levels linked to improved health outcomes.[12]

In this work, we evaluate the extract's capability to prevent Plasmodium from proliferating in infected mice as well as its capacity to alleviate these malaria-related symptoms. Our research adds to the expanding body of information about malaria natural remedies and

might pave the way for the creation of novel approaches to treatment in the battle against this formidable illness. By investigating the possibility of plant-based treatments in the fight against malaria, this study also seeks to close the knowledge gap between traditional medicine and contemporary science.

MATERIALS AND METHODS

Collection of Plant Material

Fresh Ficus trigonata stem bark was obtained from Song Local Government Area, Adamawa State. The stem bark was identified by a taxonomist at the Department of Plant Sciences, Modibbo Adama University, Yola, Adamawa State, Nigeria.

Experimental Animals

Thirty (30) adult mice, 4 weeks old, weighing 20g to 24g were procured from the University of Jos' animal breeding center located in Plateau State. The animals were then placed in well-ventilated plastic cages, and acclimatized for 7 days before the commencement of the study.

Parasite

Plasmodium berghei strain susceptible to chloroquine was gotten from National Institute of Medical Research (NIMR), Yaba, Lagos state, Nigeria.

Extraction Procedure

Maceration method of extraction as outlined by Das et al.,[13] was adopted. Fifty grams (=50g) of fresh Ficus trigonata stem bark were cleaned with water, left overnight at room temperature to dry, and then pulverized with a pestle and mortar until a powder was achieved. Exactly 500mL of methanol was used to dissolve the powdered stem bark. For five (5) days, the solution was left in place. The extracts were then filtered using a Whatman filter paper and concentrated using a rotary evaporator, the water bath was set at 40°C.

Parasite Inoculation

Through cardiac plexus puncture, parasitized erythrocytes were isolated from donor mice and mixed with trisodium citrate. Mice were intraperitoneally inoculated with 0.2mL of suspension of blood containing 10^6 - 10^7 parasitized erythrocytes on day 0.[14]

Animals Grouping and Treatment

Thirty (30) mice, separated into 5 groups of 6 mice per group. Groups A was inoculated and not treated which served as negative control. Group B was inoculated and given 10mg/kg body weight of chloroquine (standard control). Groups C, D and E were inoculated with Plasmodium berghei and treated with the extract in doses of 100, 200 and 300 mg/kg body weight respectively.[15] For each dose level the total amount of extract needed was determined using the formula:

$$Total\ amount\ of\ extract = \frac{Desired\ dose}{Concentration} \times Body\ weight\ of\ mouse$$ ....Eq. 1

Determination of Packed Cell Volume (PVC

Estimation of PCV was conducted manually. Blood samples were collected from the tail of the mice using heparinized capillary tubes in the morning as follows: before inoculation, 72 hours after inoculation, and after the first, second, and third day of treatment. Each mouse was identified with a corresponding label on both the tail and the capillary tube to ensure sample integrity. These tubes were sealed with a candle flame and organized with the sealed ends facing away from the blood to avoid contamination. A Haematocrit centrifuge was employed, and it was securely sealed before being set in motion using a timer button for a duration of 6 minutes. During this process, the blood samples separated into two distinct layers, comprising the Erythrocytes and plasma. Following centrifugation, the capillary tube containing the blood sample was carefully removed and placed onto a Hematocrit reader. Readings were then taken and recorded with precision to determine the Packed Cell Volume. PCV was determined using the formula:

$$PCV\ (\%) = (Length\ of\ red\ cell\ column)/(Total\ length\ of\ blood\ column) \times 100$$

.....Eq. 2

Determination of Parasitemia Level and Chemo-suppression activity

Parasitemia Level was assessed on four different days following the start of the treatment. To determine this, a small sample of blood from each animal's tail was placed on glass slides and allowed to air-dry. The slides were then fixed with methanol, stained using Giemsa, and examined using a 100× objective lens under a microscope. The count of red blood cells infected with parasites observed on each slide was recorded. Parasitemia level was determined using the formula below.

$$\%\ Parasitemia = (Total\ number\ of\ PRBC)/(Total\ number\ of\ RBC) \times 100$$

....Eq. 3

Where; RBC: Red Blood Cells and PRBC: Parasitized Red Blood Cells

The % parasitemia suppression of the extracts, in comparison to the untreated control, was determined [12]. Degree of suppression in parasitemia achieved by the extracts was determined using the formula:

$$\% \, Chemosuppression = \frac{MPUC - MPTG}{MPUC} \times 100 \ldots\ldots \text{Eq. 4}$$

Where; MPUC: Mean Parasitemia of Untreated Control, and

MPTG: Mean Parastemia of Treated Group

Data Analysis

The data collected were evaluated using SPSS version 27 software using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test to separate the means that are statistically different. Where $P<0.05$ was considered as statistical significance.

RESULTS

Packed Cell Volume (PCV of treated and untreated Plasmodium berghei infected mice

PCV declined steadily in negative control group from 39.57±0.48% before inoculation to 19.67±1.45% after 3 days of infection (Figure 1). In contrast, all treatment groups (receiving 100, 200, or 300mg/kg of Ficus trigonata stem bark extract) showed significantly ($p<0.05$) higher PCV levels compared to the negative control on days 1, 2, and 3 post-treatments. On day 1, PCV was 34.42±1.81%, 33.57±0.97%, and 32.86±0.63% for the 100, 200, and 300mg/kg groups, compared to only 30.67±0.62% in the negative control. This effect increased on days 2 and 3. By day 3, PCV reached 30.33±1.72%, 30.50±1.31%, and 29.43±0.37% in the 100, 200 and 300mg/kg groups respectively, compared to just 19.67±1.45% in untreated mice. The positive control group receiving the standard drug chloroquine phosphate also maintained significantly ($p<0.05$) higher PCV levels than negative controls on days 1-3, reaching 31.57±1.04% by day 3. However, on days 2 and 3, the 200 and 300mg/kg Ficus groups matched or exceeded the PCV protection demonstrated by the standard drug control (Figure 1).

Values were expressed as Mean ± SEM: n = 5.

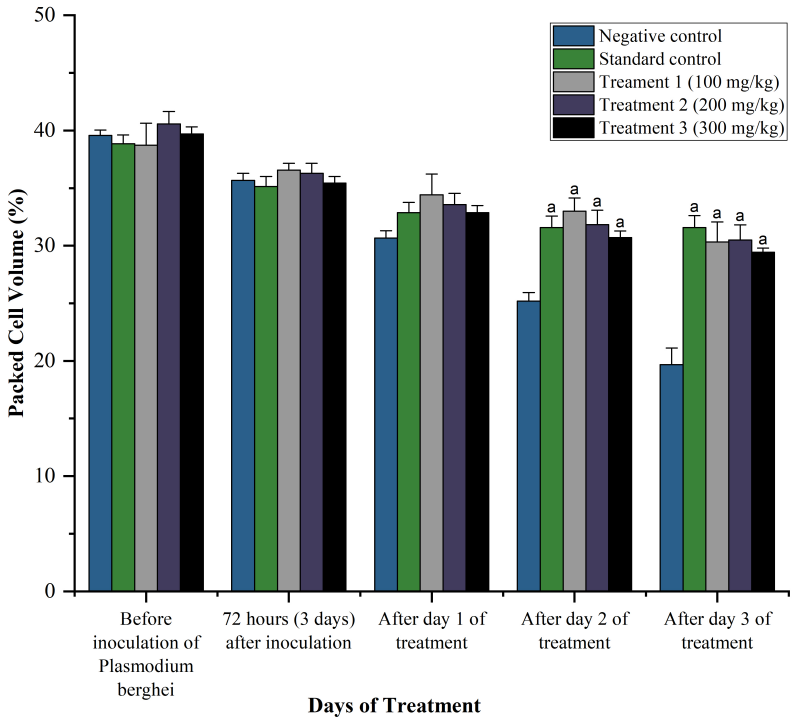

Figure 1 **Packed Cell Volume (PCV) of treated and untreatedPlasmodium berghei infected Mice**

SUPERSCRIPT = VALUES WERE SIGNIFICANTLY GREATER THAN NEGATIVE CONTROL AT P<0 05

Parasitemia level in treated and untreated Plasmodium berghei infected Mice

In the untreated negative control mice, parasitemia level rose rapidly from 14.50±1.00% at 72 hours to 33.73±1.14% by day 4 post-infection. In contrast, all F. trigonata stem bark extract treatment groups showed significantly ($p<0.05$) lower parasitemia levels compared to negative controls on days 2, 3 and 4 post-treatments. Day 2, parasitemia reached 11.14±1.26%, 9.65±1.28%, and 8.81±0.59% in the 100, 200 and 300mg/kg groups, respectively, compared to 22.46±1.13% in untreated controls. This anti-parasitic effect increased rapidly by days 3 and 4. By day 4, parasitemia was just 8.44±1.23%, 6.40±1.19% and 4.83±0.59% in the 100, 200 and 300mg/kg groups, compared to 33.73±1.14% in negative controls. The positive control group receiving chloroquine phosphate showed a similar rapid suppression of parasitemia from day 2 onwards, reaching 1.97±1.17% on day 4 (Figure 2).

Values were expressed as Mean ± SEM: n = 5.

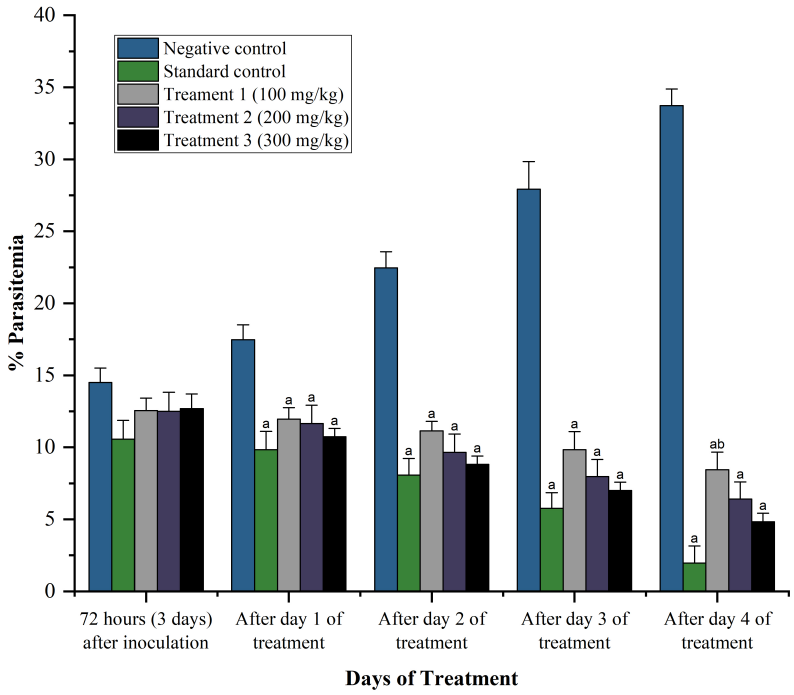

Figure 2 **Parasitemia level in treated and untreated Plasmodium berghei infected Mice**

SUPERSCRIPT = VALUES WERE SIGNIFICANTLY LESS THAN NEGATIVE CONTROL AT P<0 05

SUPERSCRIPT = VALUES WERE SIGNIFICANTLY GREATER THAN STANDARD CONTROL AT P<0 05

3.3 Chemo-suppression Effect of Methanol Extract of Ficus trigonata Stem bark in Plasmodium berghei infected mice.

On all tested days post-infection (days 1-4), the standard control drug chloroquine phosphate showed powerful chemo-suppressive activity ranging from 43.73% on day 1 to 94.16% on day 4. F. trigonata stem bark extract treatments at doses spanning 100-300mg/kg demonstrated a clear dose-dependent chemo-suppressive effect across the four-day experiment. On day 1, activity reached 31.54%, 33.31% and 38.58% for the 100, 200 and 300mg/kg F. trigonata treatment groups. This increased to 64.8%, 71.43%, and 74.94% suppressive activity by day 3 for the three increasing F. trigonata doses. By late infection on day 4, even the lowest 100mg/kg F. trigonata extract dose achieved a chemo-suppressive effect of 74.98%, approaching the 94.16% activity of the standard anti-malarial control. Higher doses further improved upon this, with 81.03% and 85.68% chemo-suppression attained by

the 200 and 300mg/kg extracts on day 4 (Figure 3).

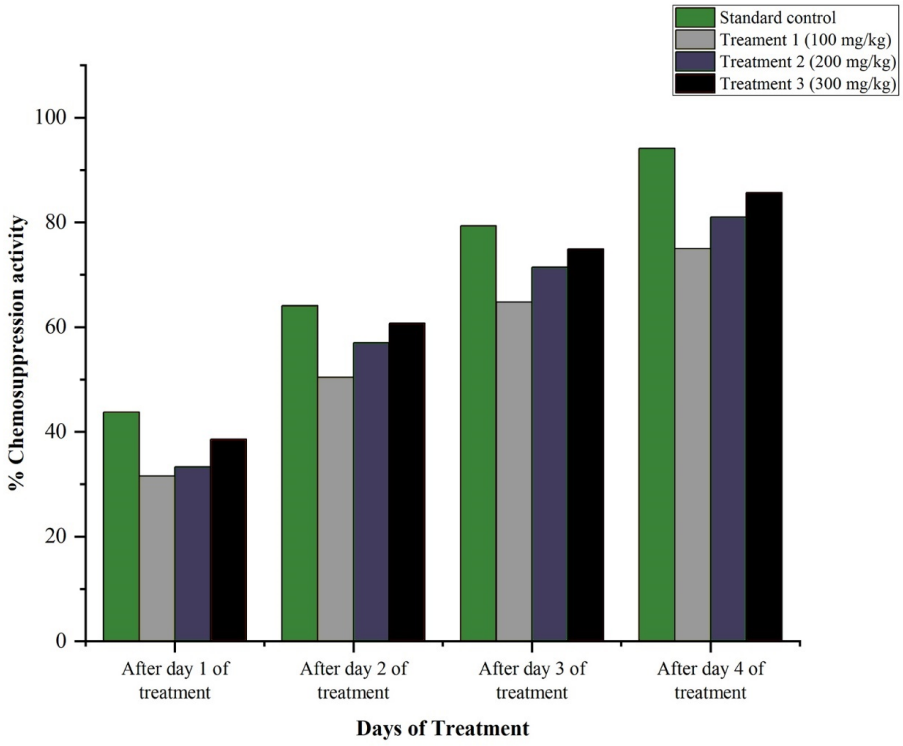

Figure 3 Chemo-suppression effect of methanol extract ofFicus trigonata stem bark in Plasmodium berghei infected mice.

DISCUSSION

In this study, it was observed that Methanol stem bark extract of Ficus trigonata had a significant ameliorative effect on PCV in Plasmodium berghei-infected mice (Figure 1). This effect was particularly prominent as the treatment duration extended. Notably, the dose (100mg/kg) displayed the most significant improvement, indicating that the extract's efficacy is positively correlated with concentration. This observation is consistent with that of Dibessa et al.,[16] , but on different medicinal plant. The decrease in PCV is a hallmark of malaria infection, as the parasite destroys red blood cells, leading to anemia and other health complications.[17]

When compared to the negative control, the extracts stopped the infected mice's PCV from dropping significantly (Figure 1). This demonstrates how the extract help avoid anemia conditions that the severe infection caused in the mice in the negative control group to appear. This may be because animals treated with extracts revealed a significant reduction in parasite burden over the course of illness. The number of parasites rose in the untreated

animals, which led to the destruction of more red blood cells and a noticeable drop in hematocrit PCV (Figure 1). This finding underscores the potential utility of methanol stem bark extract of Ficus trigonata as an adjunct therapy for malaria. The restoration of PCV levels is a crucial aspect of malaria management, as it not only improves the overall health of infected individuals but may also contribute to reducing morbidity and mortality associated with severe anemia.[18]

The parasitemia levels are a direct indicator of the severity of Plasmodium infection.[19] Lower parasitemia levels are associated with better health outcomes. The findings in the present study (Figure 2) revealed that, the extract treatment groups demonstrated a significant decrease in parasitemia levels in a manner that is time-dependent. The standard control (chloroquine phosphate) group showed the most substantial parasitemia suppression, while the extract treatment groups displayed a dose-dependent in parasitemia levels reduction. Remarkably, the highest dose of the extract (300mg/kg) effectively suppressed parasitemia, comparable to the standard control. These findings indicated that methanol stem bark extract of Ficus trigonata possessed anti-malarial activities, which align with previous studies highlighting the antiplasmodial potential of some Ficus species on multi-drug-resistant Plasmodium falciparum strain K1.[20] The extract's ability to reduce parasitemia levels suggests its potential as an alternative or adjunct antimalarial treatment.

Chemo-suppression is a vital aspect of malaria research, as it reflects the capacity of a treatment to inhibit the development and multiplication of Plasmodium parasites.[21] In this present study (Figure 3), methanol stem bark extract of Ficus trigonata exhibited notable chemo-suppression activity in Plasmodium berghei-infected mice. The extract treatment groups exhibited increasing chemo-suppression in a manner that is dose-dependent over the course of treatment. By day 4, the highest dose of the extract displayed the most substantial chemo-suppression activity of 85.68%, albeit all groups exhibited a significant reduction in parasitemia levels compared to the standard control. The findings on the anti-plasmodial activity of Ficus trigonata stem bark extract exasperate in this study are consistent with the results reported by Okon et al.,[3]. These findings suggested that methanol stem bark extract of Ficus trigonata has potential as an antimalarial agent, effectively inhibiting the growth and proliferation of Plasmodium parasites. The dose-dependent nature of this effect reinforces the idea that higher concentrations of the extract may offer enhanced efficacy which may be attributed to the plant's phytochemicals. The mechanism likely involves stimulation of the immune system to better control infection, similar to other Ficus species.[22] Disrupting heme detoxification pathways in the parasite is another possibility.[23] Extract components like flavonoids and tannins could also directly inhibit plasmodial enzymes necessary for growth.[24] Additionally, antioxidant effects help mitigate redox imbalance in malaria infection.[25] Although mechanistic insights and clinical evaluations in human trials are still needed, these findings provide a strong foundation for prioritizing F. trigonata stem bark as a promising lead for affordable antimalarial drug development programs, especially against emerging drug-resistant strains.

CONCLUSION

The study demonstrated the antimalarial potential of Ficus trigonata 300 mg/kg of body weight stem bark extract in malaria-infected mice. Administration of 300 mg/kg of body weight of extract significantly restored PCV levels and suppressed P. berghei parasitemia in a dose-dependent manner. The mechanisms that likely contributed are immunomodulation, plasmodial enzyme inhibition, metabolic interference, and antioxidant effects. These multiple actions underlie the extract's antiplasmodial properties. The results indicate phytochemical components in the extract exhibit therapeutic efficacy against malarial progression and associated anemia. This investigation highlights F. trigonata stem bark as a promising candidate for safer, affordable antimalarial agent development.

ABBREVIATIONS

MPUC: Mean Parasitemia of Untreated Control, MPTG: Mean Parasitemia of Treated Group, NIMR: National Institute of Medical Research, PCV: Packed Cell Volume, RBC: Red Blood Cells, PRBC: Parasitized Red Blood Cells, SPSS: Statistical Package for Social Sciences SEM: Standard Error of the Mean.

Acknowlegments

The authors sincerely appreciate Biochemistry department of Modibbo Adama University, Yola for allowing them to use the departmental laboratory and other necessary facilities for the success of this study.

DECLARATIONS

Authors' contributions

Conceptualization: MSJ and AUW, Funding acquisition: MSJ, YU, AUW. Data curation: MSJ and YU. Formal analysis and investigation: MSJ, YU, AUW. Methodology, software, supervision, and validation: MSJ, YU, AUW. Writing original draft, and writing of review and editing: MSJ, YU, AUW. Project administration: MSJ, YU, AUW, Resources: MSJ, YU, AUW, Visualization: MSJ and YU. All the authors review and approve the final draft.

Conflict of interest:

Authors declared that there is no conflict of interest.

Ethical approvals:

The Animal Ethics Committee at the department of Animal Science, Faculty of Agricultural science, Modibbo Adama University, Yola, gave the clearance and authorization for the use of animals in this study (MAU/FAS/AEC/AS/2023/020).

Data availability:

The supporting data of this study is always obtainable on request from the corresponding author.

Funding resources:

No funding support received for this work. The work is fully funded by the authors.

REFERENCES

1. World Health Organization. WHO World. 2020;2020. doi.org/10.1163/9789004636774_048.
2. Maiga FO, Wele M, Toure SM, Keita M, Tangara CO, Refeld RR. Artemisinin-based combination therapy for uncomplicated plasmodium falciparum malaria in mali: A systematic review and meta-analysis. Malaria Journal. 2021;20(1):356–356. doi.org/10.1186/s12936-021-03890-0.
3. Okon OE, Gboeloh LB, Ud SE. Antimalarial effect of combined extracts of the leaf of ficus exasperata and stem bark of anthocleista vogelii on mice experimentally infected with plasmodium berghei berghei (nk 65). Research Journal of Medicinal Plant. 2014;8:99–111. 10.3923/rjmp.2014.99.111.
4. Bekono BD, Ntie-Kang F, Onguéné PA, Lifongo LL, Sippl W, Fester K. The potential of anti-malarial compounds derived from african medicinal plants: A review of pharmacological evaluations from 2013 to 2019. Malaria Journal. 2020;19(1):183–183. doi.org/10.1186/s12936-020-03231-7.
5. Afifi WM, Ragab EA, Im AES, Atef A. Bioactivities and phytoconstituents of ficus trigonata. Journal of Pharmacognosy and Phytochemistry. 2014;3(4):178–84.
6. Falade MO, Akinboye DO, Gbotosho GO, Ajaiyeoba EO, Happi TC, Abiodun OO. In vitro and in vivo antimalarial activity of ficus thonningii blume (moraceae) and lophira alata banks (ochnaceae), identified from the ethnomedicine of the nigerian middle belt. Journal of Parasitology Research. 2014;p. 972853–972853. doi.org/10.1155/2014/972853.
7. Kotepui M, Phunphuech B, Phiwklam N, Chupeerach C, Duangmano S. Effect of malarial infection on haematological parameters in population near thailand-myanmar border. Malar J. 2014;13:218–218. doi.org/10.1186/1475-2875-13-218.
8. El-Hamed A, Yousef WF, Mazrou NS, Elkholy Y, El-Refaiy W, Elfeky AI, et al. Anticryptosporidium efficacy of olea europaea and ficus carica leaves extract in immunocompromised mice associated with biochemical characters and antioxidative system. Cells. 2021;10(9). 10.3390/cells10092419.
9. Girmaw F, Engidawork E. In vivo anti-malarial activity of the aqueous root extract of euclea divinorum hiern (ebenaceae) against plasmodium berghei anka. Evidence-Based Complementary and Alternative Medicine. 2022;2022:2640648–2640648. doi.org/10.1155/2022/2640648.
10. Kumar S, Kapkoti DS, Mina PR, Gupta M, Kumar R, Kumar P. Effect of liquiritigenin on chloroquine accumulation in digestive vacuole leading to apoptosis-like death of chloroquine-resistant p. Falciparum. Phytomedicine. 2023;114:154738–154738. doi.org/10.1016/j.phymed.2023.154738.

11. Hemmer CJ, Holst F, Kern P, Chiwakata CB, Dietrich M, Reisinger EC. Stronger host response per parasitized erythrocyte in plasmodium vivax or ovale than in plasmodium falciparum malaria. Tropical Medicine &amp. International Health. 2006;11(6):817–840. doi.org/10.1111/j.1365-3156.2006.01635.x.
12. Dikasso D, Makonnen E, Debella A, Abebe D, Urga K, Makonnen W. In vivo antimalarial activity of hydroalcoholic extracts from asparagus africanus lam. In mice infected with plasmodium berghei</i&gt. Ethiopian Journal of Health Development. 2007;20(2). 10.4314/ejhd.v20i2.10021.
13. Das K, Tiwari R, Shrivastava D. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of medicinal plants research. 2010;4(2):104–115. 10.5897/JMPR09.030.
14. Ii S, Emmanuel A, Nok AJ. Antimalaria effect of the ethanolic stem bark extracts of ficus platyphylla. Journal of Parasitology Research. 2011;24. doi.org/10.1155/2011/618209.
15. Anigboro AA, Avwioroko OJ, Cholu CO. Phytochemical constituents, antimalarial efficacy, and protective effect of eucalyptus camaldulensis aqueous leaf extract in plasmodium berghei-infected mice. Prev Nutr Food Sci. 2020;25(1):58–64. 10.3746/pnf.2020.25.1.58.
16. Dibessa TT, Engidawork E, Nedi T, Teklehaymanot T. Antimalarial activity of the aqueous extract of the latex of aloe pirottae berger. (aloaceae) against plasmodium berghei in mice. Journal of Ethnopharmacology. 2020;255:112763–112763. doi.org/10.1016/j.jep.2020.112763.
17. Saltarelli R, Palma F, Gioacchini AM, Calcabrini C, Mancini U, Bellis D, et al. Phytochemical composition, antioxidant and antiproliferative activities and effects on nuclear DNA of ethanolic extract from an italian mycelial isolate of ganoderma lucidum. J Ethnopharmacol. 2019;231:464–73. doi.org/10.1016/j.jep.2018.11.041.
18. PC C, editor. Pathophysiology of plasmodium falciparum-infected erythrocytes and thiol- mediated antioxidant detoxification systems; 2015. 10.4172/2470-6965.1000S1-003.
19. Mangal P, Mittal S, Kachhawa K, Agrawal D, Rath B, Kumar S. Analysis of the clinical profile in patients with plasmodium falciparum malaria and its association with parasite density. J Glob Infect Dis. 2017;9(2):60–65. 10.4103/0974-777X.201626.
20. Bwalya AG, Phiri P, Kaiser M, Tasdemir D. Evaluation of the in vitro antimalarial and malaria prophylactic activity of eight ficus species. Planta Med. 2011;77(12):16–16. 10.1055/s-0031-1282139.
21. Araka A, Ayibadinipre J. Potency of ficus exasparata leaf extract on albino mice infected with plasmodium berghei berghei. Greener Journal of Medical Sciences. 2023;13(1):69–73.
22. Yang X, Guo JL, Ye JY, Zhang YX, Wang W. The effects of ficus carica polysaccharide on immune response and expression of some immune-related genes in grass carp, ctenopharyngodon idella. Fish Shellfish Immunol. 2015;42(1):132–139. doi.org/10.1016/j.fsi.2014.10.037.

23. Chandana M, Anand A, Ghosh S, Das R, Beura S, Jena S. Malaria parasite heme biosynthesis promotes and griseofulvin protects against cerebral malaria in mice. Nature Communications. 2022;13(1):4028–4028. doi.org/10.1038/s41467-022-31431-z.
24. Tajbakhsh E, Kwenti TE, Kheyri P, Nezaratizade S, Lindsay DS, Khamesipour F. Antiplasmodial, antimalarial activities and toxicity of african medicinal plants: A systematic review of literature. Malaria journal. 2021;20(1):1–50. doi.org/10.1186/s12936-021-03866-0.
25. Kratchanova M, Denev P, Ciz M, Lojek A, Mihailov A. Evaluation of antioxidant activity of medicinal plants containing polyphenol compounds. Comparison of two extraction systems. Acta Biochim Pol. 2010;57(2):229–263. 20532255.