



Anti-malarial and Therapeutic Potential of Ethanolic Leaf and Root Extracts of *Alstonia boonei* Against *Plasmodium berghei* Infection in Mice

Chidiebere A. Otuu^{1*}, Rose N. N. Obiezue², Samuel S. Eke³, Hadijah Usman-Yamman⁴, Innocent C. Ekuma⁵, Emmanuel. O. Udeh⁶, Ada Q. A. Otuu⁷

¹Parasitology and Public Health Unit, Department of Animal and Environmental Biology, Federal University Oye-Ekiti, Ekiti State, Nigeria

²Parasitology and Public Health Unit, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State, Nigeria

³Department of Biology, Air Force Institute of Technology, Kaduna, Kaduna State, Nigeria

⁴Department of Public Health Science, Newgate University, Minna, Niger State, Nigeria

⁵Department of Biomedical Engineering, Alex Ekwueme Federal Teaching Hospital, Abakaliki, Ebonyi State, Nigeria

⁶Centre for Integrated Health Programs, Wuse 2, Federal Capital Territory, Abuja, Nigeria

⁷Department of Pharmacy, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State, Nigeria

Abstract

Introduction: The main objective of this research was to investigate the antimalarial therapeutic potential of the ethanolic extract of the leaves and roots of *Alstonia boonei*, a West African medicinal plant that is used for the traditional treatment of malaria, fever, and other parasitic diseases. There is a need to scientifically evaluate various plants utilized in the ancient treatment of diseases as they might be potential sources of new modern drugs; thus, the importance of this study is obvious.

Methods: The extracts were subjected to *in vivo* antimalarial tests in mice in order to determine their efficacy against malaria infection. These materials exhibited dose-dependent antimalarial activity ($P < 0.05$), as indicated by suppressive and curative effects on mice infected with doses of 100, 200, and 400 mg/kg body weights of the *Plasmodium berghei* malaria parasite.

Results: The suppressive test of the extracts revealed a significant dose-dependent early infection suppression at $P < 0.05$.

Conclusion: Based on the results, the plant should be further examined to analyze its bioactive compounds and position it as a potential source of new and novel molecules for antimalarial drug development.

Keywords: Malaria, Therapeutic Potential, *Alstonia boonei*, *Plasmodium berghei*, Mice

Received: May 15, 2023, Accepted: June 10, 2023, ePublished: June 29, 2023

Introduction

Using chemicals extracted from different plant sources for the treatment of many diseases is still pronounced in many countries, mainly due to the lack of adequate healthcare and the drug delivery system (1). Thus, such means of treating diseases still play important roles in such countries (2,3). However, there is a need for scientific studies on such plants to ascertain their safety, dosage, and efficacy as alternatives to orthodox medicines and evaluate their chemical composition for potential drug discoveries (4–6).

Today, most of the new synthetic drugs are made from plants that were extensively used by the old traditional doctors (7, 8). *Alstonia boonei* is one of the medicinal plants that grows and is abundant in Nigeria and other African countries (9). Different parts of the

stem, leaves, and roots of this plant are traditionally utilized to treat many different parasitic diseases, such as malaria (10). Many phytochemical compounds have been isolated from *A. boonei*, and some of them possess an array of medicinal properties, including antimalarial activities (11,12).

Recent research has been geared up to investigate the efficacy of traditional antimalarial medicines and position them for the potential development of new antimalarial molecules with new mechanisms against the malaria parasite (13,14). This development may be attributed to the increasing prevalence of malaria in endemic areas of the disease (15-17). Today, with the discovery of new drugs and effective compounds against malaria, treatment and control measures, as well as prevention and eradication of malaria, have become easier (18).



Materials and Methods

Animals for the Experiment

Experiments on laboratory animals were performed according to international laboratory standards. Inbred albino mice weighing between 20–22 g were used in this study.

Parasite Strain

Artesunate-sensitive *Plasmodium berghei* NK65 strain was utilized in the current study (19).

Antimalarial Screening of the Extract

Suppressive and curative antimalarial tests were used to demonstrate the malaria therapeutic efficacy of the extract in albino mice experimentally infected with *P. berghei*.

Measurement of Therapeutic Activity

The therapeutic activities of the ethanolic leaf and root extracts were evaluated on parasitic infections of *P. berghei* in mice using the modified techniques (20,21). Another set of male and female mice was randomly selected and infected with 10^7 *P. berghei* on the first day. Seventy-two hours later, a set of mice were treated with doses of 100, 200, and 400 mg kg⁻¹ body weights of the extracts. The negative control group set of mice was given 5 mLkg⁻¹ distilled water, while the positive control group set of mice was treated with 5 mgkg⁻¹ artesunate. Daily treatment was performed for five consecutive days. The parasitaemia level of this parasite was determined by preparing thin blood smears stained with Giemsa and studying them under a light microscope.

Results

Antimalarial Tests

The suppressive test of the extracts represented a significant dose-dependent early infection suppression at $P < 0.05$. The findings of the inhibitory effects of the extracts are presented in Tables 1 and 2.

The curative test of the extracts showed a dose-dependent reduction in parasitaemia in the established infections with *P. berghei* (Tables 3 and 4).

Discussion

The results from the suppressive and curative tests of the ethanolic leaf extract of *A. boonei* revealed that the lowest inhibition of parasitaemia was at the lowest administered dose of 100 mg/kg body weight, while the highest inhibition of parasitaemia was at the highest administered dose of 400 mg/kg body weight. This result corresponds to that of some researchers, focusing on the *in vivo* antimalarial activity of the ethanolic leaf extract of *A. boonei* against early infection (suppressive effect) and established infection (curative effect) in the experimental mouse model (22–25). Their result also demonstrated a

Table 1. Suppressive Activity of the Ethanolic Leaf Extract of *Alstonia boonei* and Chloroquine in Infected Mice With *Plasmodium berghei*

Therapeutic Compounds	Inhibition (%)
DW 5 mLkg ⁻¹	0.00 ± 0.00 ^a
Extract 100 mgkg ⁻¹	42.47 ± 1.21 ^b
Extract 200 mgkg ⁻¹	54.68 ± 0.55 ^c
Extract 400 mgkg ⁻¹	68.35 ± 1.27 ^d
Artesunate 5 mgkg ⁻¹	92.83 ± 1.10 ^e

Note. DW: Distilled water.

Table 2. Suppressive Activity of the Ethanolic Root Extract of *Alstonia boonei* and Artesunate in Mice Infected With *Plasmodium berghei*

Therapeutic Compounds	Inhibition (%)
DW 5 mLkg ⁻¹	0.00 ± 0.00 ^a
Extract 100 mgkg ⁻¹	43.86 ± 0.58 ^b
Extract 200 mgkg ⁻¹	56.84 ± 1.15 ^c
Extract 400 mgkg ⁻¹	65.42 ± 0.64 ^d
Artesunate 5 mgkg ⁻¹	94.56 ± 0.64 ^e

Note. DW: Distilled water.

Table 3. Curative Activity of the Ethanolic Leaf Extract of *Alstonia boonei* and Artesunate in Mice Infected With *Plasmodium berghei*

Therapeutic Compounds	Inhibition (%)
DW 5 mLkg ⁻¹	0.00 ± 0.00 ^a
Extract 100 mgkg ⁻¹	61.96 ± 0.57 ^b
Extract 200 mgkg ⁻¹	65.57 ± 0.01 ^c
Extract 400 mgkg ⁻¹	69.38 ± 0.64 ^d
Artesunate 5 mgkg ⁻¹	94.53 ± 0.64 ^e

Note. DW: Distilled water.

Table 4. Curative Activity of the Ethanolic Root Extract of *Alstonia boonei* and Artesunate in Mice Infected With *Plasmodium berghei*

Therapeutic Compounds	Inhibition (%)
DW 5 mLkg ⁻¹	0.00 ± 0.00 ^a
Extract 100 mgkg ⁻¹	56.99 ± 0.58 ^b
Extract 200 mgkg ⁻¹	62.48 ± 0.63 ^c
Extract 400 mgkg ⁻¹	67.39 ± 0.64 ^d
Artesunate 5 mgkg ⁻¹	96.28 ± 0.64 ^e

Note. DW: Distilled water.

high level of antimalarial effects in the suppressive and curative tests with parasitaemia (% + standard error of mean [SEM]) of 2.80 + 0.86 and 1.60 + 0.26 for the suppressive and curative effects, respectively, which is in line with the results of this study. In an *in vivo* study on the antiplasmodial activity of the *A. boonei* leaf extract against established *P. berghei* NK65 infection in mice, some researchers found that the optimal percentage parasitaemia of 6.0 + 0.32 (SEM) was obtained in mice treated with the highest dose (26,27). This result is similar to the findings in the studies performed by some researchers, representing significant mean percentage inhibition of parasitaemia in their antimalarial tests (28–30).

Recent research conducted by some scientists has shown that medicinal plants have less side effects and drug residues in the body tissues of consumers compared to synthetic antiparasitic drugs (30).

Conclusion and Recommendations

The findings of this research determined that the extracts of some medicinal plants have good and effective therapeutic properties for the treatment and control of Plasmodium, the cause of malaria. Future studies should focus on discovering new chemical compounds and antimalarial drugs.

Author's Contribution

Conceptualization: Chidiebere A. Otuu.

Data curation: Rose N. N. Obiezue.

Formal analysis: Samuel S. Eke.

Funding acquisition: Hadijah Usman-Yamman.

Investigation: Innocent C. Ekuma.

Methodology: Emmanuel. O. Udeh.

Resources: Ada Q. A. Otuu.

Software: Rose N. N. Obiezue.

Supervision: Chidiebere A. Otuu.

Validation: Hadijah Usman-Yamman.

Visualization: Chidiebere A. Otuu.

Writing—original draft: Chidiebere A. Otuu.

Competing Interests

The authors of the article have no conflict of interests.

References

- Adebayo JO, Krettli AU. Potential antimalarials from Nigerian plants: a review. *J Ethnopharmacol.* 2011;133(2):289-302. doi: [10.1016/j.jep.2010.11.024](https://doi.org/10.1016/j.jep.2010.11.024).
- Afolabi OJ, Abejide AE. Antiplasmodial activities of *Morinda lucida* (Benth) and *Alstonia boonei* (De wild) in mice infected with *Plasmodium berghei*. *Bull Natl Res Cent.* 2020;44(1):85. doi: [10.1186/s42269-020-00342-8](https://doi.org/10.1186/s42269-020-00342-8).
- Asuzu IU, Anaga AO. Pharmacological screening of the aqueous extract of *Alstonia boonei* stem bark. *Fitoterapia.* 1991;63(5):411-7.
- Awe SO, Opeke OO. Effect of *Alstonia congensis* on *Plasmodium berghei* in mice. *Fitoterapia.* 1990;61(3):225-9.
- Babamale OA, Iyiola OA, Adeyemi SB, Sulaiman AF, Abdulkareem AO, Anifowoshe AT, et al. Comparative studies of genotoxicity and anti-plasmodial activities of stem and leaf extracts of *Alstonia boonei* (De Wild) in malaria-infected mice. *Niger J Parasitol.* 2017;38(2):192-7.
- Bankole AE, Adekunle AA, Sowemimo AA, Umebese CE, Abiodun O, Gbotosho GO. Phytochemical screening and in vivo antimalarial activity of extracts from three medicinal plants used in malaria treatment in Nigeria. *Parasitol Res.* 2016;115(1):299-305. doi: [10.1007/s00436-015-4747-x](https://doi.org/10.1007/s00436-015-4747-x).
- Batista R, Silva Ade J Jr, de Oliveira AB. Plant-derived antimalarial agents: new leads and efficient phytomedicines. Part II. Non-alkaloidal natural products. *Molecules.* 2009;14(8):3037-72. doi: [10.3390/molecules14083037](https://doi.org/10.3390/molecules14083037).
- Bekono BD, Ntie-Kang F, Onguéné PA, Lifongo LL, Sippl W, Fester K, et al. The potential of anti-malarial compounds derived from African medicinal plants: a review of pharmacological evaluations from 2013 to 2019. *Malar J.* 2020;19(1):183. doi: [10.1186/s12936-020-03231-7](https://doi.org/10.1186/s12936-020-03231-7).
- Bello IS, Oduola T, Adeosun OG, Omisore NO, Raheem GO, Ademosun AA. Evaluation of antimalarial activity of various fractions of *Morinda lucida* leaf extract and *Alstonia boonei* stem bark. *Glob J Pharmacol.* 2009;3(3):163-5.
- Builders ML. African traditional antimalarials: a review. *Pharm Chem J.* 2017;4(6):87-98.
- Cragg GM, Newman DJ. Biodiversity: a continuing source of novel drug leads. *Pure Appl Chem.* 2005;77(1):7-24. doi: [10.1351/pac200577010007](https://doi.org/10.1351/pac200577010007).
- Marie-Esther DU, Concilia OC, Chioma U, Chimaobi KF, Austin O. In vivo antimalarial and cytotoxicity activity of ethanolic stem bark of *Petersianthus macrocarpus* and leaf of *Alstonia boonei* in experimental mice model. *Int J Curr Microbiol Appl Sci.* 2013;2(12):354-68.
- Dondorp AM, Nosten F, Yi P, Das D, Phyto AP, Tarning J, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med.* 2009;361(5):455-67. doi: [10.1056/NEJMoa0808859](https://doi.org/10.1056/NEJMoa0808859).
- Dondorp AM, Yeung S, White L, Nguon C, Day NP, Socheat D, et al. Artemisinin resistance: current status and scenarios for containment. *Nat Rev Microbiol.* 2010;8(4):272-80. doi: [10.1038/nrmicro2331](https://doi.org/10.1038/nrmicro2331).
- Ebiloma G, Amlabu E, Atanu FO, Amlabu W, Rhoda OA. Effect of the aqueous extracts of *Alstonia boonei* on the haematological profiles of mice experimentally infected with the chloroquine-sensitive strain of *Plasmodium berghei* NK-65. *Hematologia.* 2012;1(1):11-8.
- Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery: efficacy models for compound screening. *Nat Rev Drug Discov.* 2004;3(6):509-20. doi: [10.1038/nrd1416](https://doi.org/10.1038/nrd1416).
- Idowu OA, Soniran OT, Ajana O, Aworinde DO. Ethnobotanical survey of antimalarial plants used in Ogun State, Southwest Nigeria. *Afr J Pharm Pharmacol.* 2010;4(2):55-60.
- Imam AA, Ezema MD, Muhammad IU, Atiku MK, Alhassan AJ, Idi A, et al. In vivo antimalarial activity of solvents extracts of *Alstonia boonei* stem bark and partial characterization of most active extract(s). *J Pharm Res Int.* 2017;19(2):1-10. doi: [10.9734/jpri/2017/36236](https://doi.org/10.9734/jpri/2017/36236).
- Iyiola OA, Tijani AY, Lateef KM. Antimalarial activity of ethanolic stem bark extract of *Alstonia boonei* in mice. *Asian J Biol Sci.* 2011;4(3):235-43. doi: [10.3923/ajbs.2011.235.243](https://doi.org/10.3923/ajbs.2011.235.243).
- Pratyush K, Misra CS, James J, Dev LM, Veetil AK, Thankamani V. Ethnobotanical and pharmacological study of *Alstonia* (Apocynaceae)-a review. *J Pharm Sci Res.* 2011;3(8):1394-403.
- Lawal B, Shittu OK, Kabiru AY, Jigam AA, Umar MB, Berinyuy EB, et al. Potential antimalarials from African natural products: a review. *J Intercult Ethnopharmacol.* 2015;4(4):318-43. doi: [10.5455/jice.20150928102856](https://doi.org/10.5455/jice.20150928102856).
- Madhiri R, Vijayalakshmi G. A review on phytochemical composition and pharmacological aspects of the genus *Alstonia*. *PharmaTutor.* 2018;6(1):50-5.
- Omoya F, Oyebola TF. Antiplasmodial activity of stem bark and leaves of *Alstonia boonei* (De Wild). *J Microbiol Exp.* 2019;7(5):241-5.
- Onifade OF, Maganda V. In vivo activity of ethanolic extract of *Alstonia boonei* leaves against *Plasmodium berghei* in mice. *J Worldw Holist Sustain Dev.* 2015;1(4):60-8.
- Shibeshi MA, Kifle ZD, Atnafie SA. Antimalarial drug resistance and novel targets for antimalarial drug discovery. *Infect Drug Resist.* 2020;13:4047-60. doi: [10.2147/idr.s279433](https://doi.org/10.2147/idr.s279433).
- Tajbakhsh E, Kwenti TE, Kheyri P, Nezaratzade S, Lindsay DS, Khamesipour F. Antiplasmodial, antimalarial activities and toxicity of African medicinal plants: a systematic review of literature. *Malar J.* 2021;20(1):349. doi: [10.1186/s12936-020-03231-7](https://doi.org/10.1186/s12936-020-03231-7).

- 10.1186/s12936-021-03866-0.
27. Uzor PF, PrasastyVD, Agubata CO. Natural products as sources of antimalarial drugs. *Evid Based Complement Alternat Med.* 2020;2020:9385125. doi: [10.1155/2020/9385125](https://doi.org/10.1155/2020/9385125).
 28. Garedaghi Y, Bahavarnia SR. Repairing effect of *Allium cepa* on testis degeneration caused by *Toxoplasma gondii* in the rat. *Int J Womens Health Reprod Sci.* 2014;2(2):80-9. doi: [10.15296/ijwhr.2014.12](https://doi.org/10.15296/ijwhr.2014.12).
 29. Gharadaghi Y, Shojaee S, Khaki A, Hatf A, Ahmadi Ashtiani HR, Rastegar H, et al. Modulating effect of *Allium cepa* on kidney apoptosis caused by *Toxoplasma gondii*. *Adv Pharm Bull.* 2012;2(1):1-6. doi: [10.5681/apb.2012.001](https://doi.org/10.5681/apb.2012.001).
 30. World Health Organization (WHO). Update on Artemisinin Resistance. 2012. <http://www.who.int/malaria/publications/atoz/arupdate042012.Pdf>. Accessed 2023.

© 2023 The Author(s); This is an open-access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.