International Journal of Basic & Clinical Pharmacology
Okwesileze CN et al. Int J Basic Clin Pharmacol. 2022 Jul;11(4):296-301
http://www.ijbcp.com
pISSN 2319-2003 | eISSN 2279-0780

DOI: https://dx.doi.org/10.18203/2319-2003.ijbcp20221594

Original Research Article

Antiplasmodial activity of the ethanol extract of *Dacryodes edulis* leaf in *Plasmodium berghei* infected mice

Chikezie N. Okwesileze[1], Godwin C. Akuodor[2]*, Casimir C. Ofor[3], Daniel O. J. Aja[3], Gideon A. Ibiam[4], Cajetan E. Ilo[2]

[1]Department of Pharmacy, University of Calabar Teaching Hospital, Calabar, Nigeria
[2]Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Science, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nigeria
[3]Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, College of Medicine, Ebonyi State University, Abakaliki, Nigeria
[4]Department of Medical Laboratory Science, Faculty Health Science and Technology, Ebonyi State University, Abakaliki, Nigeria

Received: 17 March 2022
Revised: 13 April 2022
Accepted: 14 April 2022

***Correspondence:**
Dr. Godwin C. Akuodor,
Email: goddyakuodor@yahoo.com

ABSTRACT

Background: Malaria treatment in Africa is increasingly becoming difficult resulting from *Plasmodium falciparum* resistant to available antimalarial agents. Therefore, evaluating plants used in traditional medicines can lead to alternative treatment against malaria.
Methods: This study evaluated the antiplasmodial activity of ethanol extract of *Dacryodes edulis* in chloroquine sensitive *Plasmodium berghei* NK65 strain in mice. The 4-day suppressive test, repository and curative effect against established rodent malaria infection models were assayed in this study. The oral acute toxicity test and phytochemical screening were also carried out on the ethanol leaf extract.
Results: The ethanol leaf extract of *Dacryodes edulis (D. edulis)* at varying doses of 100 mg/kg, 200 mg/kg, 400 mg/kg significantly ($p<0.05$) exhibited dose-dependent decrease in parasitaemia at suppressive, repository and curative studies. Chloroquine 10 mg/kg significantly ($p<0.05$) decreased parasitaemia levels in the three models of malaria infections. The antiplasmodial activity of the leaf extract is comparable with the standard drug. The extract was found to be non-toxic acutely in mice.
Conclusions: The findings show that *D. edulis* ethanol leaf extract demonstrated high antiplasmodial activity in a dose-dependent fashion. Thus, supporting claims of the plant traditional therapeutic importance for malaria treatment, and can be developed as an alternative therapy against the disease.

Keywords: *Dacryodes edulis*, Leaf extract, Herbal medicine, Antiplasmodial activity, Mice

INTRODUCTION

Malaria is a widespread parasitic infection ravaging different parts of the world, especially within tropical and subtropical regions. It is estimated that malaria infects over 500 million humans annually and causes more than 2 million deaths in children.[1] More than 40% of the world population lives in endemic environments, especially in equatorial areas that favour the growth of mosquitoes.[1,2] The most devastating manifestation of malaria in man is caused by *Plasmodium falciparum*. The parasite has resisted virtually all antimalarial drugs in use, making resistant strains spread fast.[3,4] Resistance to these agents has become a major challenge facing malaria control in developing countries. More recently, attention generated by drug-resistant *Plasmodium falciparum* strains has led to

growing interest in research for antimalarial remedies. Natural products from plants, have maintained an important source of new agents against malaria.[5,6] Therefore, there is an urgent need to thoroughly examine these natural habitats from different communities possibly through discoveries that might be useful to mankind.[7]

However, in traditional medicine, herbal agents have been prescribed as an intervention in malaria treatment. Different traditionally used plants exhibit pharmacological properties with potential for therapeutic applications in the treatment of malaria and its disorders.[8] Hence, studies on plants together with their beneficial effects could provide leads, for the synthesis of essential active metabolites.

D. edulis is a shade loving, dioecious, evergreen tree found in the humid tropical zone of the non-flooded forest.[9,10] It is indigenous to the Gulf of Guinea and widely cultivated in other tropical parts of Africa. This plant which belongs to the family Burseraceae possesses many medicinal and nutritional properties. In Nigeria, when is in season, the fruit pulp which is contained in a pod is traditionally consumed raw or after tenderization in hot water, hot ash or roasted.[11] Sometimes, it may be enjoyed with roasted or boiled corn.[12]

D. edulis is commonly known as 'African pear', it is known as eben among the Efik people and orumu in Benin both in South-South, Nigeria, ube in Igbo (South-Eastern Nigeria), elemi in Yoruba, South West and safou in French.[13,14] The plant has long been used in the traditional medicine of some African countries to treat various ailments such as wounds and scars, malaria, fever and skin diseases.[15-17] The leaf extract and its secondary metabolites have been found to show biological activities such as antimicrobial, antioxidant and anti-sickle cell anaemia.[18-21]

However, the interest in *D. edulis* was justified by its application in the treatment of different diseases. Therefore, this study aimed aim to evaluate the antiplasmodial activity of the ethanol leaf extract of *D. edulis* in mice.

METHODS

Preparation of plant extract

The leaves of *D. edulis* were collected from the African peer tree in Calabar, Cross River State, Nigeria. The plant was identified by a taxonomist at the herbarium unit, Department of Botany, University of Calabar, Nigeria. Five hundred grams (500 g) of leaves were washed and shed-dried in the laboratory for 7 days at room temperature pulverized to a coarse powder, macerated for 48 h in 1.5 l of 98 per cent ethanol and left overnight. The mixture was then filtered and evaporated to semi-solid mass using a rotary evaporator (Brichi, Germany) and subsequently dried in a beaker on a water bath to give a dark resinous mass.

Phytochemical analysis

The ethanol leaf extract of *D. edulis* was subjected to qualitative phytochemical analysis to determine the secondary metabolites following the procedure as described by Billmary et al.[22]

Acute toxicity studies

The acute oral toxicity of the leaf extract was invested using Lorke's (1983) method.[23] This study was in two phases and mice were deprived of food overnight before extract administration. In phase1, three groups of three animals per group were used. The extract was orally given in increasing doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg. Animals treated were monitored for signs of toxicity in the first 4 hours and after 24 hours. With the absence of death after 24 hours, phase 2 was introduced. Three groups of one mouse were each given the extract orally at doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg. The animals were then observed for signs of toxicity and mortality for 24 hours and 48 hours respectively.

Animals

Male and female mice (18-22 g) obtained from Animal House, Department of Pharmacology, Faculty of Allied Medical Sciences, University of Calabar, were used for the study. The males were separated from the females and maintained under standard laboratory conditions of 25–30 ^{o}C, 12-hour light and 12-hour darkness cycles. The mice were maintained in clean cages with sawdust, which was replaced every two days and had access to a pellet diet, water when needed. The study was approved by the institutional ethics committee of (University of Calabar) (Approval No FAREC/PA/012PA3116). The animals were randomly distributed into experimental groups. National Institute of Health Guide for care and use of laboratory animals was used. [24]

Anti-plasmodial study

Plasmodium berghei

The NK65 sensitive chloroquine *P. berghei* infected mice were sourced from National Institute for Medical Research (NIMR), Lagos. They were kept in the Pharmacology Department Animals House unit, University of Calabar. The parasites were maintained by a continuous intraperitoneal passage in mice every four days, and the re-infected mice were used for the study.[25]

Inoculums

Infected red blood cells from the donor mouse were collected through cardiac puncture after anaesthesia with chloroform. Microscopic examination of smeared and stained thin blood film was used to establish parasitaemia. Each mouse was intraperitoneally passaged with 0.2 ml (1×10^{7}) parasitized erythrocyte suspension in saline from the donor mouse.

Suppressive test

Thirty adult mice of both sexes were selected and passaged. After six hours, the infected mice were randomly divided into five groups of six mice each which include three test groups receiving graded doses of 100 mg/kg, 200 mg/kg and 400 mg/kg respectively, the ethanol leaf extract of *D. edulis.*

The *P. berghei* control group received 20 ml/kg normal saline, while mice in the chloroquine diphosphate control group were administered with 10 mg/kg. These animals were treated orally for 4 days (D_0–D_3). On day 5 (D_4), the films were prepared from the tail blood of each mouse, and parasite concentration was examined microscopically, counting the parasitized red blood cells on 1000 red blood cells in 10 different fields. Inhibition of parasitaemia was then evaluated.[5]

Prophylactic study

This study was carried out according to the methods described by Essien et al,[6] Thirty albino mice of both sexes selected for this study were grouped into 5 of 6 mice per cage. Group 2-4 were treated orally with graded doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg of the ethanol leaf extract for four days (D_0-D_3); whilst groups 1 and 5 received 20 ml/kg of normal saline and 10 mg/kg of chloroquine diphosphate respectively. On the last day of treatment (D_4), mice in all groups were injected intraperitoneally with *P. berghei* infected erythrocyte suspension. After 72 hours, films were prepared (as earlier described) and examined microscopically.

Curative test

Thirty (30) mice of both sexes were intraperitoneally passaged with standard inocula of 1×10^7 *Plasmodium berghei* infected erythrocytes for 72 hours. Thereafter, they were randomly grouped into 5 with 6 mice per group and treated daily with different doses of the extract (100 mg/kg, 200 mg/kg and 400 mg/kg) respectively for 3 days. *P. berghei* infected control group received 20 ml/kg of normal saline, while mice in the chloroquine diphosphate control group received 10 mg/kg. All administrations were by oral route. Films were later prepared and viewed to determine the parasite density. Mortality was monitored daily for mean survival time (MST) and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse in the extract-treated and control groups throughout the follow-up period (D_0-D_{29}).[27]

Statistical analysis

Data were expressed as means±SEM and analyzed with a statistical package for social sciences (SPSS version 26) by using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Results were considered significant at $p<0.05$.

RESULTS

Phytochemical screening

Preliminary phytochemical screening of the ethanol extract of *D. edulis* leaf showed that alkaloids, saponins, glycosides, polyphenol, tannins, flavonoids, reducing sugars were present.

Acute toxicity

There were no signs of toxicity observed after oral administration of the ethanol extract, indicating that the acute toxicity test was greater than 5000 mg/kg in mice. Thus, the experimental doses used were relatively safe in mice.

Suppressive response

The leaf extract showed suppressive activity in a dose-dependent manna. Doses used exhibited significant ($p<0.05$) dose-dependent antiplasmodial activity. *D. edulis* ethanol extract caused 78%, 83% and 88% antiplasmodial activity respectively, while chloroquine produced 92% chemosuppressive activity (Table 1).

Table 1: Suppressive activity of the ethanol leaf extract of *D. edulis* in mice infected from *P. berghei.*

Drug	Dose (mg/kg)	Mean parasite density	% Suppress-ion
Normal saline (ml/kg)	20	32.75±1.42	-
D. edulis	100	7.25±0.28	78[a]
	200	5.65±0. 33	83[a]
	400	3.87±0.24	88[a]
Chloroquine	10	2.55±0.31	92[a]

Results are expressed as mean±SEM; (n=6); [a]significantly different from control at $p<0.05$.

Prophylactic response

The ethanol extract exhibited a dose-related effect at different doses used. Doses of 100 mg/kg, 200 mg/kg and 400 mg/kg significantly ($p<0.05$) prophylactically halted replication of *P. berghei* parasites at 77%, 85% and 93% respectively, while chloroquine 10 mg/kg showed 93% parasites inhibition (Table 2).

Curative response

The extract exhibited a significant dose-dependent reduction in parasite density. The doses of *D. edulis* ethanol leaf extract (100 mg/kg, 200 mg/kg and 400 mg/kg) exhibited significant ($p<0.05$) effect against established *P. berghei* infection comparable to the effect exhibited in chloroquine treated group; whilst in the negative group, there was a consistent increase in the blood parasites (Table 3). The survival rate among the mice also reflected dose-

dependent response and showed that the extract significantly ($p<0.05$) cleared the parasites in established infection. In no drug-treated control group, death was observed in mice from day 9, and by day 12 all mice in that group died. But the extract-treated mice survived above 22 days. In addition, few mice in 200 mg/kg and 400 mg/kg of the extract-treated survived the 30-day observation period, whereas chloroquine treated recorded no mortality.

Table 2: Prophylaxis activity of the ethanol leaf extract of *D. edulis* in mice infected from *P. berghei*.

Drug	Dose (mg/kg)	Mean parasite density	% Suppress-ion
Normal saline (ml/kg)	20	30.25±1.15	-
D. edulis	100	6.85±0.33	77[a]
	200	4.58±0.11	85[a]
	400	3.48±0.29	89[a]
Chloroquine	10	2.10±0.22	93[a]

Results are expressed as mean±SEM; (n=6); [a]significantly different from control at $p<0.05$.

Table 3: The curative activity of the ethanol leaf extract of *D. edulis* in mice infected from *P. berghei*.

Drug	Dose mg/kg	Mean parasite density	Parasite density (D7)
Normal saline (ml/kg)	20	30.25±4.58	33.66±0.23
D. edulis	100	30.15±0.75	10.78±0.59[a]
	200	29.65±0.51	8.70±0.26[a]
	400	28.87±0.33	6.30±0.62[a]
Chloroquine	10	28.75±0.31	3.14±0.44[a]

Results are expressed as mean±SEM; (n=6); [a]significantly different from control at $p<0.05$

Table 4: Mean survival time in days of ethanol leaf extract of *D. edulis* against *P. berghei* in mice during curative study.

Treatment	Dose (mg/kg)	Mean survival time in days
Control	20	10.10±0.96
D. edulis	100	22.45±1.65[a]
	200	27.33±0.68[a]
	400	29.43±0.18[a]
Chloroquine	10	29.52±0.00[a]

Results are expressed as mean±SEM; (n=6); [a]significantly different from control at $p<0.05$.

DISCUSSION

Malaria is one of the most prevalent diseases in the tropics with high mortality. However, with the current increase in parasite drug resistance, there is an urgent need to look for alternative agents for malaria treatment. The ethanol leaf extract of *D. edulis* showed antiplasmodial activity against *P. berghei* infection in mice as shown by percentage parasite inhibition. The extract decreased the parasite density in mice during the four-day suppressive test which suggests the leaf extract suppressive effect on the blood stage of the parasite comparable to that of standard antimalarial drug, chloroquine. *D. edulis* extract exhibited dose-dependent activity, as the dose increased antiplasmodial activity also significantly increased.

The extract also exerted a significant repository effect in mice. This effect was however lower in groups that received low doses. This activity may probably be due to the short duration of action of the ethanol extract caused by rapid metabolism and so the parasite could not be cleared. The ethanol leaf extract of *D. edulis* also significantly exhibited curative activity in established infection. The antiplasmodial activity of the leaf extract is consistent with the traditional application of the plant in herbal medicine against malaria infection in South-Eastern Nigeria. The rodent method of malaria which was employed in the present study for the prediction of potent antimalarial activity of *D. edulis* leaf extract was also used for conventional antimalarial drugs such as chloroquine, lumefantrine and artemisinin derivatives.[28] *P. berghei* are used in the prediction of treatment outcomes, thus, it was an appropriate parasite for this study. Since *P. berghei* was sensitive to chloroquine, it was used as the referenced drug in this study. Chloroquine is clinically used for suppressive, prophylactic and curative treatment of malaria, except for resistant strains of *P. falciparum*.[29] This agent destroys plasmodia by preventing the digestion of haemoglobin, and blocking the source of the parasite amino acids, or by inhibiting hem polymerase to prevent the production of hemozoin, a protective medium against autolysis. Although chloroquine showed higher suppressive, preventive and curative activities by its decrease in the parasite density, *D. edulis* leaf extract also exhibited similar antiplasmodial activity however to a lower level. More so, the leaf extract also enhanced the mean survival period of treated mice especially the 400 mg/kg treated extract group. The antiplasmodial activity of the leaf extract might be due to the presence of secondary metabolites variously implicated in the antiplasmodial activity of different medicinal plants.[27] In this study, the leaf extract of *D. edulis* did not show any toxic effect because doses up to 5000 mg/kg caused no death or alter the behaviour of the tested mice. Natural products with the observed activities have been reported to provide relief for patients with malaria attacks.[8]

CONCLUSION

The findings of this study support the ethnomedicinal application of this plant in the treatment of malaria and its disorders. More study is needed on *D. edulis* to determine the active component that generates antiplasmodial activities. Further studies on the purification of the leaf extract to isolate the bioactive metabolites and their structures must be elucidated.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Ritter JM, Lewis LD, Mant TGK, Ferro A. A textbook of Clinical Pharmacology and Therapeutics, 5th Edition, Hodden Education Publisher, London. 2008;364.
2. de Ridder S, van der Kooy F, Verpote R. Artemisia annua as a self-reliant treatment for Malaria in developing countries. J Ethnopharmacol. 2008;120:303-14.
3. World Health Organization Malaria. 2012. Fact sheet no. 94. Available at: http://www.who.int/mediacentre/factsheets/fs094/en/. Accessed on 05 February 2022.
4. Plowe CV, Djimde A, Bouare M, Doumbo O, Wellens TE. Pyrimethamine and proguanil resistance-conferring mutation in Plasmodium falciparum dihydrofolate reductase: polymerase chain reaction methods for surveillance in Africa. Am J Trop Med Hyg. 1995;52:565-68.
5. Kaushik NK, Bagavan A, Rahuman AA, Mohanakrishna D, Kamaraj C, Elango G. Antiplasmodial potential of selected medicinal plants from Eastern Ghats of South India. Exp Parasitol. 2013;134:26-62.
6. Lombardino JG, Lowe JA. The role of the medicinal chemist in drug discovery-then and now. Nat Rev Drug Discov. 2004;3:853-62.
7. Akuodor GC, Ajoku GA, Ezeunala MN, Chilaka KC Asika EC. Antimalarial potential of the ethanolic leaf extract of Pseudocedrala kotschyi. J Acute Disease. 2015;23-7.
8. Akuodor GC, Ezeonu TC, Essien AD, Asika EC, Chilaka KC Nwachukwu DC, et al. In vivo antiplasmodial activity of Bombax buonopozense root bark aqueous extract in infected by Plasmodium berghei. J Tradit Chin Med. 2017;37(4):431-5.
9. Aidu M, Erhabor JO, Towuru GE. Antimicrobial effects of the chloroform and ethanolic leaf extracts of Dacryodes edulis (G. Don) H. J. Lam, Garcinia Kola Heckel and Chrysophyllum Albidum G. Don on some test isolates. Med Sci. 2013;1(3):63-6.
10. Leakey RRB, Tchoundjeu Z, Schreckenberg K, Schacleton SE, Shackleton CM. Agroforestry tree products (AFTPS): Targeting poverty and enhanced livelihoods. Int J Agric Sustainability. 2005;3(1):1-23.
11. Isaac IO, Ekpa OD. Minerals and anti-nutrients in two varieties of African pear (Dacryodes edulis). J. Food Technology. 2009;7:106-10.
12. Iyawe HOT, Osagie VE, Esekheigbe A and Azih MC. Nutrient composition and effect of seed extracts of Africa black pear (Dacyodes edulis) on rats. Asian J Plant Science. 2007;6:878-80.
13. Kengue J, Tchuenguem F, Adewusi H. Towards the improvement of safou (Dacryodes edulis), population and reproduction Biology. Afri J Biotec. 2002;12:73-84.
14. Nwokeji E, Nwufo M, Ibe A. Investigation on the uses and fungal diseases of African pear (Dacryodes edulis) in Imo state, Nigeria. Nig J Plant Protect. 2005;22:9-16.
15. Omonhinmin A. Ethnobotany of Dacryodes edulis (G. Don) in Southern Nigeria 2: Practices and application among Igbo speaking people. J Ethnobotany Res Application. 2012;10:175-84.
16. Egharvba RKA, IKhatua I. Ethno medical uses of plants in the treatment of various skin diseases in Ovia North Easth, Edo State Nig Res J Agric Biol Sci. 2008;4(1):58-64.
17. Okwu D, Nnamdi F. Evaluation of the chemical composition of Dacryodes edulis and Raphia hookeri Mann and Wendl exudates used in Herbal medicine in South East Nigeria. Afri J Tradit Complem Altern Med. 2008;5(2):194-200.
18. Obame L, Edou P, Bassole I, Koudou J, Agnaniet H, Eba F, Traore A. Chemical composition, antioxidant, antimicrobial properties of essential oil of Dacryodes edulis from Gabon. Afri J Microbiol Res. 2008;2:148-52.
19. Nguefack EC. Hypoglycemic, Hypolipidemic and antioxidant activity of some Cameroonian Medicinal plants. Lyon France. 2009;49.
20. Agbor G, Kuate D, Oben J. Medicinal plants can be good sources of antioxidants, case study in Cameroon. Park J Biological Sci. 2007;100:537-44.
21. Mpiana P, Tshibangu D, Shetonde O, Ngboluak. In Vitro antidrepanocytary activity (anti-sickle cell anaemia) of some Congolese plants. Phytomedicine. 2007;14:192-5.
22. Billmary C, Janne R, Luccro M, Maria C. Preliminary Phytochemical Screening of Pirmenta racemosa Var. racemose (myrtaceae) from Tachira-venezula. Pharmacology online. 2015;1:121-5.
23. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983;54:275-87.
24. NIH. Guide for the Care and Use of Laboratory Animals. 8th ed. Bethesda MD. National Institutes of Health. 2011;82-3.
25. Akuodor G, Amos G, Essien A, David-oku E. Akpan J, Ezeokpo B. Antimalaria potency of the leaf extract of Aspilia Africana (Pears). Asian Pac J Trop Med. 2012;5:126-9.
26. Essien AD, Essiet GA, Akuodor GC, Nwoboo NN, Akpan JL, Utsalo SJ. Evaluation of anti plasmochial effect of the ethanolic leaf extract of Salacia lehmbachii on Plasmodium berghei infected mice. Eur J Biol Res. 2017;7(2):124-30.
27. Abdulrazak N, Asiya UI, Usman NS, Unata IM, Farida A. Antiplasmodial activity of ethanolic extract of root and stem back of Cassia sieberiana DC on mice. J. Intercult Ethnopharm. 2015;4(2):96-101.
28. Builder MI, Wannang NN, Ajoku GA, Builder PF, Orisadipe A, Aguiyi JC. Evaluation of the antiplasmodial potential of Vernonia ambigua kotschu

and Peyr(Asteraceae). Int J Pharmacol. 2011;7:238-47.
29. Mbah CC, Akuodor GC, Anyalewechi NA, Iwuanyanwu TC, Osunkwo UA. In vivo antiplasmodial activities of aqueous extract of Bridelia ferruginea stem bark against Plasmodium berghei berghei in mice. Pharm Biol. 2012;50:188-92.

Cite this article as: Okwesileze CN, Akuodor GC, Ofor CC, Aja DOJ, Ibiam GA, Ilo CE. Antiplasmodial activity of the ethanol extract of Dacryodes edulis leaf in Plasmodium berghei infected mice. Int J Basic Clin Pharmacol 2022;11:296-301.