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Phytochemical Screening and Antiplasmodial Activity of Ethanolic Bark Extract of *Khaya grandifoliola* in Swiss Albino Mice Infected with *Plasmodium berghei* NK65

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Authors' contributions

This work was carried out in collaboration between all authors. Author AAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AAA, EOD and DM managed the analyses of the study. Authors AAA and EOD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Irrespective of the decreased in incidence and prevalence, malaria remains a major public health problem. Evolution and spread of resistance to the available antimalarial drugs endanger all the recent gains in malaria control. This issue makes the development of novel drugs a necessity. The key source in search of such drugs is medicinal plants (*Khaya grandifoliola*). *Khaya grandifoliola* is use for management of malaria, but no scientific investigations have been carried out to substantiate the usage. Thus, this study assessed the bioactive components and antiplasmodial activity of ethanolic bark extract of *K. grandifoliola*. Standard methods were employed to determine the bioactive components of the bark extract. Twenty four (24) mice were randomly selected into six

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groups of four mice each (group 1, 2, 3, 4, 5 and 6) for antiplasmodial activity. The *Plasmodium berghei* infected groups were treated with *K. grandifoliola* extract with 0.2 mL of 200 mg/kg, 400 mg/kg and 600 mg/kg body weight respectively. Group 1 (negative control) was infected with *P. berghei*and administered with 0.2 mL of normal saline, Group 2 (positive control) were treated with 0.2 mL of 5 mg/kg body weight of chloroquine while group 3 (normal control) was not infected and administered with 0.2mL of normal saline for four consecutive days. Phytochemical Screening showed alkaloids, saponins, tannins and anthraquinone. The extract treated groups 4, 5 and 6 revealed decrease in percentage parasitaemia comparedwith group 1 (infected and not treated). The parasitaemia reduction was high in group 6 (600 mg/kg). The significant decrease ($P<0.05$) in percentage parasitaemia was dose and time dependent. This result indicates that *K. grandifoliola* has a promising antiplasmodial activity and it could be considered as a potential source to develop new antimalarial agents.

Keywords: Khaya grandifoliola; Plasmodium berghei; antiplasmodial activity; phytochemical screening; albino mice.

1. INTRODUCTION

Malaria is a mosquito-borne infectious disease affecting humans and other animals, caused by parasitic protozoa belonging to the *plasmodium* type. These parasites are spread to people through the bite of infected female *Anopheles* mosquitoes [1]. *Plasmodium berghei* belongs the phylum Apicomplexa, class Aconoidasida, subclass Haemosporidiasina, order Chromatorida, suborder *Laveraniina*, family *Plasmodiidae*, genus *Plasmodium* and the species *P. berghei* to the family of Plasmodiidaes a protozoan that causes malaria in certain rodents [2]. Originally *P. berghei* was isolated from thicket r Central Africa, *P. berghei* is one of four *plasmodium* species that have been described in African murine rodents, the others being *P. chabaudi*, *P. vinckei*, and *P. yoelii*. due to its ability to infect rodents and relative ease of genetic engeneering, *P. berghei* is popularly model organism for study of human malaria [2]. According to [1], the five species of *Plasmodium* that cause malaria in human are: *P. falciparum*, *P*. *vivax*, *P*. *ovale*, *P. malariae* and *P*. *knowles*i. *P. falciparum* is the most common type which cause malaria disease in humans. *P.knowles*i, this species of the *Plasmodium* rarely causes disease in human. In the work of Caraballo [3], malaria is most commonly transmitted by infected saliva of female *Anopheles* mosquito into uninfected person's blood. Malaria symptoms are usually accompanied with fever, tiredness, shivering, vomiting, headache, yellow skin, seizures, coma, or death. In several cases, the symptoms usually begin 10- 15 days after being bitten with *P. falciparum.* The risk of this disease can be reduced by preventing mosquito bites through the use of mosquito nets and insects' repellents or with mosquito control measure such as spraying insecticides and standing water Carabello [3]. Several medications are available to prevent malaria in travellers to areas where the disease is common [1]. Occasional doses of the combination medication Sulfadoxinepyrimethamine are recommended in infants and after the first trimester of pregnancy in areas with high rates of malaria. The recommended treatment for malaria is a combination of antimalaria medications [1]. Several years ago, antimalaria drug such as chloroquine, amodiaquine, mefloquine were effectively used to treat malaria, however the evolution and spread of resistance to these drugs are of major public health concern, hence, studies on medicinal plants could come out with useful information for the production of important active and non-toxic compounds as antimalarials. *Khaya grandifoliola* also called African Mahogany, Benin Mahogany, Large- leaved Mahogany, or Senegal Mahogany is a species of plant in the Malieaceae family, order Sapindales, genus *Khaya* and the species *K. grandifoliola*. It is found in Benin, the Democratic Republic of the Congo, Ivory Coast, Ghana, Guinea, Nigeria, Sudan, Togo, and Ugabda [4]. In many malaria endemic countrie like the tropics, the extract of the *Khaya grandifoliola* is used as an antimalaria herbal remedy. Recent studies show that 90% of malaria cases around the world come from sub-Saharan Africa. People in these areas resort to medicinal plants for treatment because alternative medicinal resources are often unavailable. The bark and seeds of *K. grandifoliola* are the most common parts used for treatment and are extracted by infusion or decoction. The extracts have proven to fight against the *P. falciparum* parasite, one of the vectors of malaria in humans [5]. This research work is aimed at determining the phytochemical

component of the ethanolic bark extracts of *K. grandifoliola* and its antiplasmodial effect in swiss albino mice infected with *Plasmodium berghei* NK 65.

2. MATERIALS AND METHODS

2.1 Plant Bark Collection

The fresh stem bark of *K*. *grandifoliol*a also known as oganwo in Yoruba and Africa Mahogany in English Language were collected from Iboropa Akoko Ondo State South Western Nigeria, it was identified and authenticated in the Department of Biology, the Federal University of Technology, Akure, Nigeria. The voucher specimen number of the plant Bio/ FUTA/ 700 was left in the herbarium of the Federal University of Technology, Akure, Ondo State, Nigeria.

2.2 Extraction and Phytochemical Screening of the Bark

Bankole [6] was adopted for the extraction. The bark plant was washed chopped into pieces with mortar and pestle and sun dried for two to three weeks, then stored in an air tight bottle before the analysis. Five hundred grams (500 g) of the grinded powder was soaked into 2.5 litres of 75% ethanol, stirred and left for 72 hours with continues shaking. The mixture was filtered using a whatman filter paper (pore size 0.7 µm), the reddish brown was concentrated into dryness in a ceramic container using water bath at 40°C to obtain extract free of solvent and was refrigerated at -4°C for further studies.

2.3 Determination of Phytochemicals

The method of Bankole [6], was used to carry out phytochemical screening of powdered samples.

2.4 Preparation of Bark Extracts Dosages

The dosages of the extract administered to the mice were prepared by dissolving 1, 2 and 3 g of the extract in 5 mL of distilled water each in sterile universal bottle to obtain 200, 400 and 600 mg/kg respectively [7].

2.5 Assemblage of Experimental Mice

Mice weighing between 19-23 g were obtained from animal house, Institute of Advance Medical Research and Training (IMRAT), University College Hospital (UCH) Ibadan. Mice were housed in plastic cages with saw dust beddings. The animals were fed with pellets, clean water and acclimatised for 7 days at room temperature 29°C -30°C before the commencement of the experiment. *Plasmodium berghei* NK65 in a donor mouse was obtained from IMRAT.

2.6 Grouping of Animals

A sum of 16 mice were used for acute toxicity test and was grouped into four each, while a total of twenty four (24) mice were divided into six groups of four mice per group for antiplasmodium activity. Group 1 (not treated), group 2 (chloroquine treated group), group 3 (normal control), groups 4, 5 and 6 (extract treated groups) (4).

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2.7 Acute Toxicity

Acute toxicity test of the stem back extract was carried out using Irungu [5]. Each mouse was respectively administered orally with 0.2 ml (200, 400, 800, 1000 mg/kg body weight) of *Khaya grandifoliola* ethanolic back extract. The mice were observed for seven days for mortality, body weakness, hyper activity; reduce activity, licking paw, salivation, inactiveness and death.

2.8 Preparation of Inoculum

Donor mouse containing 20% parasitaemia was autnaesthesised with chloroform 0.2 mls of the parasite was withdrew from the infected mouse and serially diluted with sterile 4.8 mls of normal saline to obtain 1_x10^7 *Plasmodium berghei* infected erythrocyte [4].

2.9 *In vivo* Antiplasmodial Activity of the Extract

Antiplasmodial activity of the extract was carried out using the peter's 4 days suppressive test as adopted by Dada and Oloruntola [6]. Twenty-four (24) mice were randomly distributed into six groups of four mice per group. They were infected intravenously with 0.2 ml of 1_x10^7 standard inoculum of chloroquine sensitive *P. berghei.* Four hours after infection, 0.2 mL of 200, 400 and 600 mg/kg body weight of bark extracts were administered orally to group 4, 5 and 6 respectively as treatment dose once daily for four consecutive days. Group 2 which is the positive control were treated with 0.2 mL of 5 mg/kg body weight of chloroquine, group 1 were

given 0.2 ml of normal saline which is the negative control and group 3 which is normal control received 0.2 ml of normal saline but were not infected with *P. berghei.*

2.10 Determination of Parasitaemia

The method described by Bankole [6] was used. On day 5 of the experiment, 2 drops of blood sample were respectively collected onto grease free microscopic slides from the mice caudal vein. Thick and thin blood smear were made and allowed to air-dry. The air-dried films were fixed with 75% ethanol for 2-3 minutes, after which they were stained with 10% Giemsa stain for 15 minutes. The blood smear samples were rinsed with buffer distilled water PH 7.2. The parasitaemia was determined by counting the part of the film where the white cells and parasites were evenly distributed. Using oil immersion objectives (X100), white blood cells were counted systematically; hand tally counter was used to count at the same time to know the parasite present in the fields.

% Parasitemia = (Number of parasitised RBC X100 / Total Number of RBC examined)

2.11 Statistical Analysis

All data were expressed as mean±S.E.One-way analysis of variance was used to analyse data. P<0.05wasconsidered significant difference between means (Duncan's multiple range test).

3. RESULTS

3.1 Phytochemical Screening of the Ethanolic Bark Extract of *K. grandifoliola*

Phytochemical screening of ethanolic bark extract of *K. grandifoliola* revealed the presence of alkaloids, saponins, tannins, anthraquinone, flavonoid and carbohydrate.

Table 1. Photochemical screening of ethanolic bark extract of *K. grandifoliola*

Plant name *K. grandifoliola*	Result
Alkaloid	+
Flavonoid	-
Saponins	+
Phenols	+
Carbohydrate	+
Anthraquinone	+

3.2 Acute Toxicity of the Ethanolic Bark Extract of *K. grandifoliola*

No sign of toxicity such as body weakness, hyperactivity, salivation, jumping, reduced activity, licking paw, convulsion, in the mice and no mortality was recorded for all the doses tested. The result indicated that the LD_{50} is greater than 1000 mg/kg body weight.

3.3 The Effect of Bark Extract of *K. grandifoliola*on Body Weight of Mice Before and After Infection with *Plasmodium berghie*

The body weight of the group 1 and 2 (infected untreated and infected treated with 5 mg/kg chloroquine) showed no significant difference P>0.05 after 4 days of treatment (Fig. 1). However, the mice treated with 400 mg/kg (group 4) had the highest decreased in body weight compared with those treated with 200 mg/kg and 600 mg/kg (group 4 and 6) after 4 days of treatment. Mice in group 3 (normal saline) had increased body weight.

The Fig. 1 revealed the body weight of mice infected with *P*. *berghei* and treated with bark extract of *K*. *grandifoliola* before and after infection. The Fig. 1 showed decreased in the body weight of the groups treated with the plant bark extract except group 3 which is normal that is group that was not infected with *P*. *berghei.*

4. DISCUSSION

This study revealed that bark extract of *K*. *grandifoliola* contains bioactive compounds such as alkaloids, saponins, flavonoids, tannins and glycosides, this agrees with [7].

The outcome of the acute toxicity test in this study revealed no observable behavioural signs of toxicity and mortality suggest that the ethanolic extract of K. *grandifoliola* may not be toxic to the experimental mice at this dosage used and therefore be considered to be safe for consumption in treatment of malaria fever and other ailments.

The decreased in the body weight of both the infected and untreated mice (negative control) agrees with [8] that weight decreased in the infected mice with *Plasmodium berghei* might be attributed to the occurrence of anorexia, that is loss of appetite which is usually associated with malaria infection.

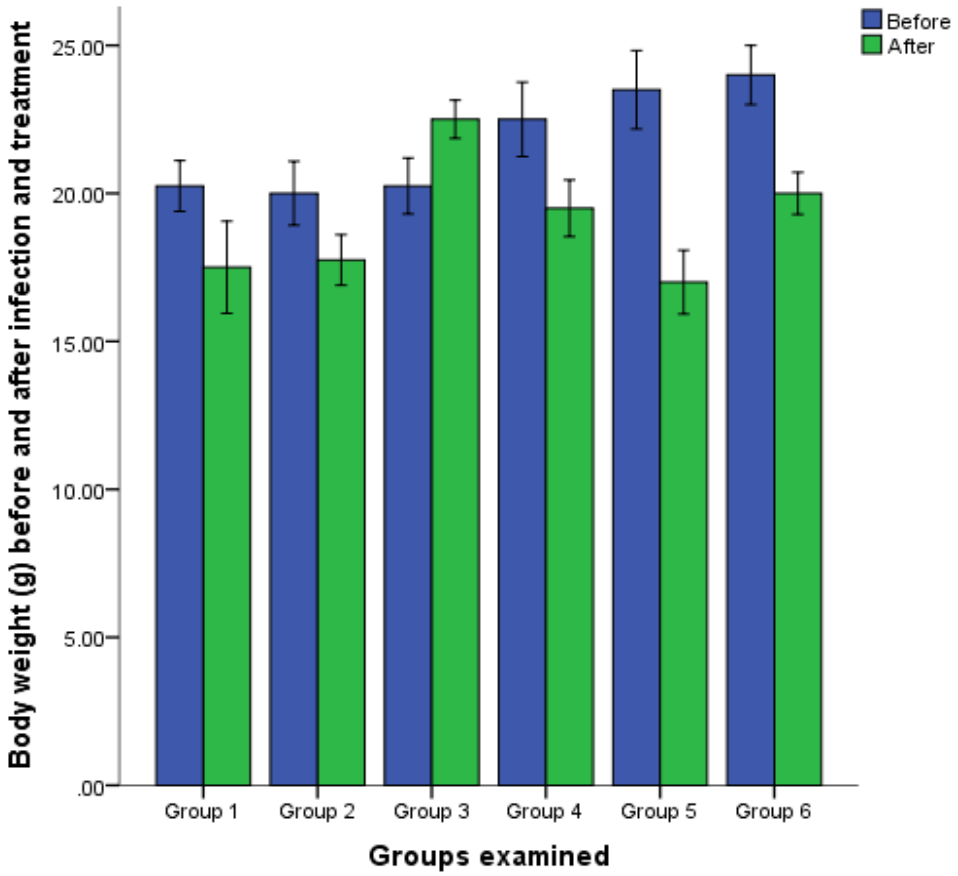

Fig. 1. Body weight before and after infection and treatment with the bark extract of the plant *K. grandifoliola*

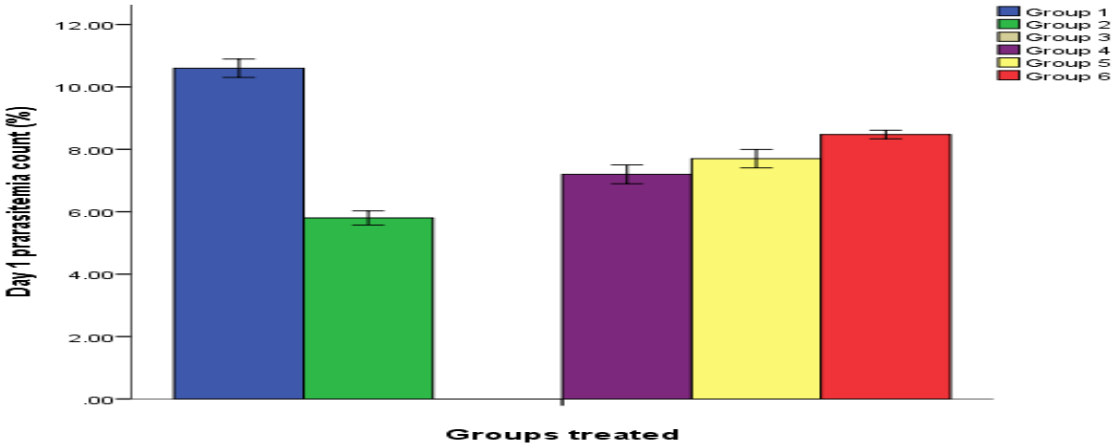

Fig. 2. Parasitemia count (%) for Day 1

Group 1: P. berghei + 0.2 ml normal saline, group 2: P. berghei + 5 mg/kg body weight Chloroquine, group 3: 0. 2 ml normal saline, group 4: P. berghei+ 200 mg/kg body weight bark extract, group 5: P. berghei + 400 mg/kg body weight bark extract and; group 6: P. berghei + 600 mg/kg body weight bark extract

The dose dependent antiplasmodial activity showed in this study corroborates with Deharo and Ginsburg [9] who observed parasites clearing with ethanolic extract of medicinal plants such as *K. grandifoliola* as attributed to the presence of phytochemical constituent like Alkaloids and others in thestudied extract.

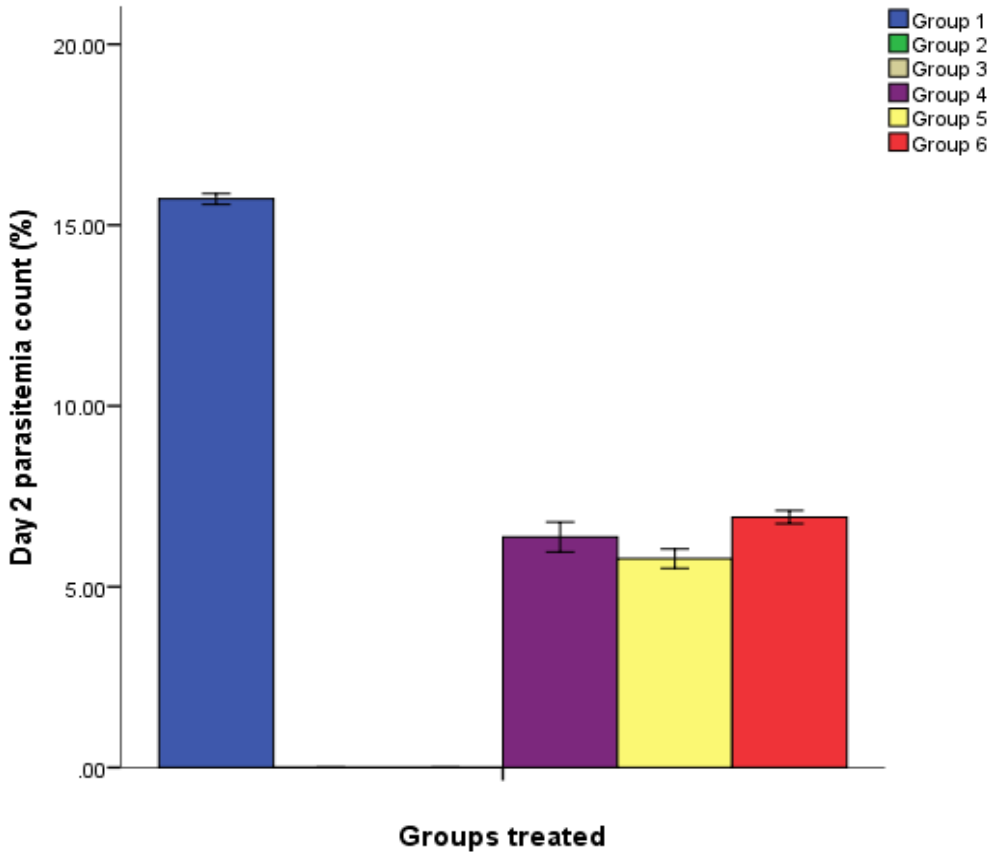

Fig. 3. Parasitemia count (%) for Day 2

Group 1: P. berghei + 0.2 ml normal saline, group 2: P. berghei + 5 mg/kg body weight Chloroquine, group 3: 0. 2 ml normal saline, group 4: P. berghei+ 200 mg/kg body weight bark extract, group 5: P. berghei + 400 mg/kg body weight bark extract and; group 6: P. berghei + 600 mg/kg body weight bark extract

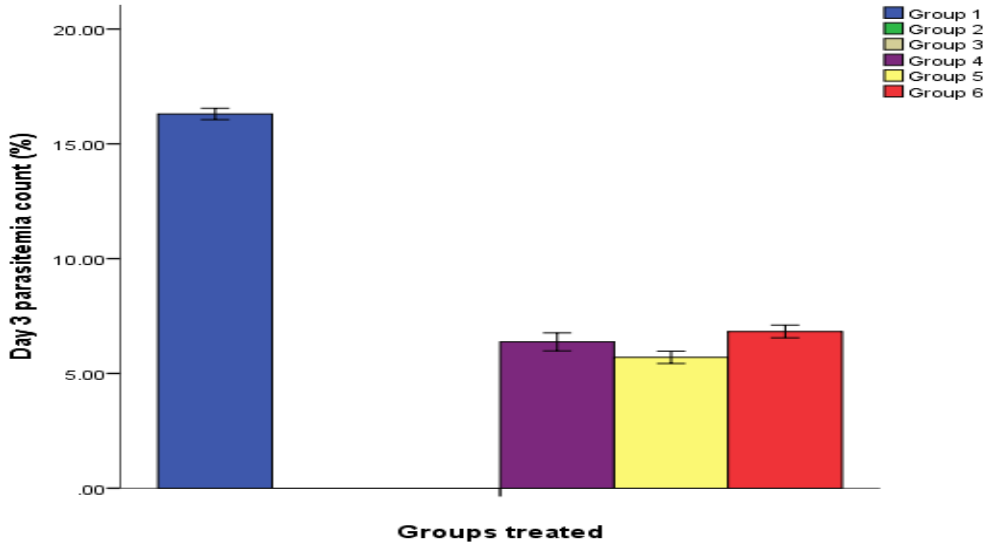

Fig. 4. Parasitemia count (%) for Day 3

Group 1: P. berghei + 0.2 ml normal saline, group 2: P. berghei + 5 mg/kg body weight Chloroquine, group 3: 0. 2 ml normal saline, group 4: P. berghei+ 200 mg/kg body weight bark extract, group 5: P. berghei + 400 mg/kg body weight bark extract and; group 6: P. berghei + 600 mg/kg body weight bark extract

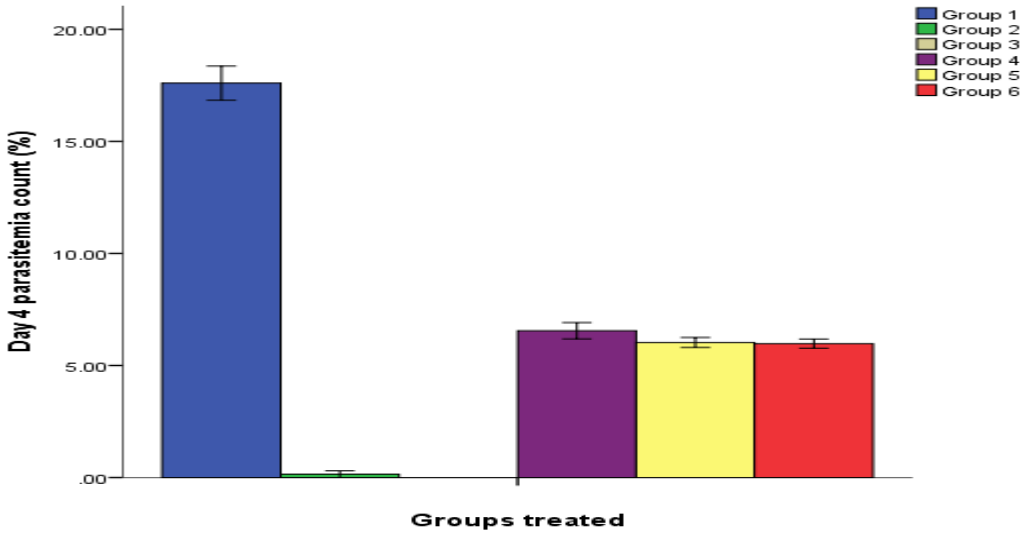

Fig. 5. Parasitemia count (%) for Day 4

Group 1: P. berghei + 0.2 ml normal saline, group 2: P. berghei + 5 mg/kg body weight Chloroquine, group 3: 0. 2 ml normal saline, group 4: P. berghei+ 200 mg/kg body weight bark extract, group 5: P. berghei + 400 mg/kg body weight bark extract and; group 6: P. berghei + 600 mg/kg body weight bark extract

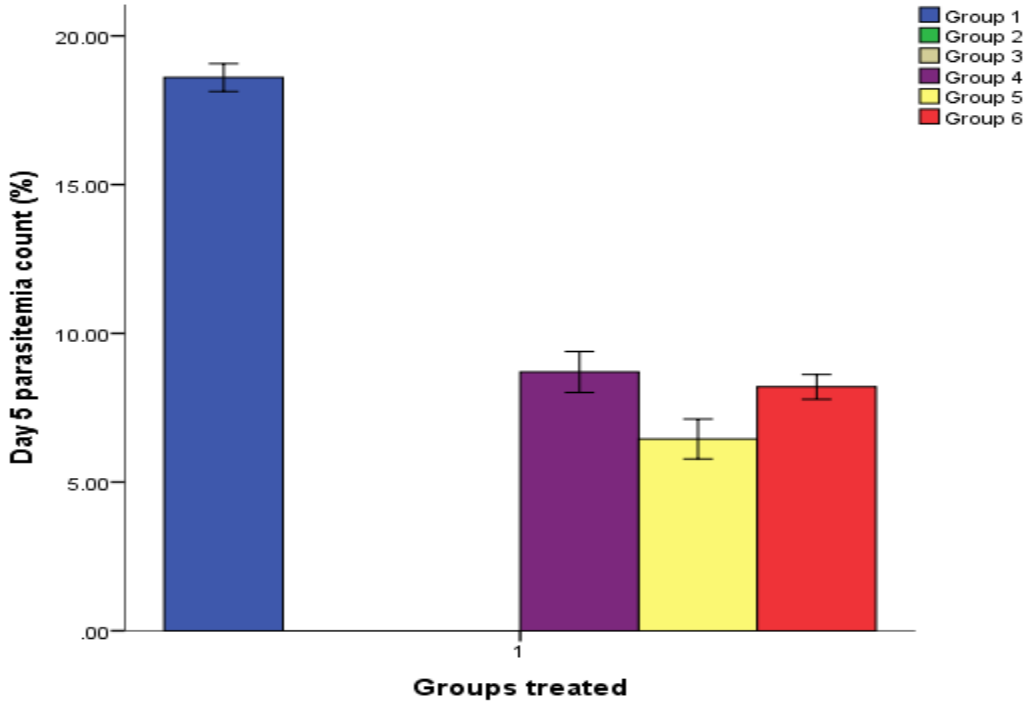

Fig. 6. Parasitemia count (%) for Day 5

Group 1: P. berghei + 0.2 ml normal saline, group 2: P. berghei + 5 mg/kg body weight Chloroquine, group 3: 0. 2 ml normal saline, group 4: P. berghei+ 200 mg/kg body weight bark extract, group 5: P. berghei + 400 mg/kg body weight bark extract and; group 6: P. berghei + 600 mg/kg body weight bark extract

5. CONCLUSION

The results obtained from this study revealed that the ethanolic bark extract of the *Khaya grandifoliola* significantly P<0.05 produced low parasitemia and improved in *Plasmodium berghei* infected mice which can be used in the treatment of malaria. Also, the bark extract is capable of reducing parasitaemia in the infected experimented mice.

ETHICAL APPROVAL

The whole experimental management, handling and care were approved by the Research and Ethical Committee of Microbiology Department School of Science, The Federal University of Technology, Akure, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Malaria Report. General Switzarland: WHO. 2014;32-42. ISBN: 978-92-156483-0.1565523
2. Junaid Quaizim Olawale, Khaw Loke Tim, Mahmud Rohela, Ong Kien Chai, Lau Yee Ling, Borade Prajakta Uttam, Liew Jonathan Wee Kent, Sivanandam Sinnadurai, Wong Kum Thong, Vythilingam Indra. Pathogenesis of *Plasmodium berghei* ANKA infection in the gerbil (*Meriones unguilatus*) as an experimental model for severe malaria parasite. 2017;24:38.
   DOI: 10.1051/parasite/2017040
3. Caraballo H. Emergency department management of mosquito-borne illness: Malaria, and West Nile virus. Emergency Medicine Practice. 2014;16(5).
   (Archieved from Original on 2016-08-01)
4. Bickii J, Njifutie N, Foyere JA, Basco LK, Ringwald P. *In vitro* antimalaria activity of Limonoids from *Khaya grandifoliola* C.D.C (Maliaceae). J Ethnopharmacol. 2000; 69(1):27-33.
   PMID: 10661881
5. Odugbemi TO, Akinsulire OR, Aibinu IE, Fabeku PO. Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, Southwest Nigeria. African Journal of Traditional, Complentary and Alternative Medicines. 2007;4(2):191-8.
   DOI: 109.4314/ajtcam.v4i2.31207
6. Bankole AE, Adekunle AA, Sowemimo AA, Umebese CE, Abiodun O, Gbotosho GO. Phytochemical screening and *in vivo* antimalaria activity of extracts from three medicinal plants used in malaria treatment in Nigeria. Parasitology Research. 2016;115:299-305.
7. Tor-Anyiin TA, Akpuaka MU, Oluma HO. Phytochemical and antimicrobial studies on stem bark extract of *Sterculia setigera*, Del. Afr J Biotechnol. 2011;10(53):11011-11015.
8. Bashir GW, Hasballah K, Chong WC, Talib H, Yam MF, Jabbarzarere TH, Othman F, Moklas MA, Abdullah WO, Ahmed Z. *Plasmodium berghei* ANKA infection in ICR mice as model of cerebral malaria. Ian Journal of Parasitology. 2012;7(4):62-74.
9. Deharo EA, Ginsburg H. Call for using natural compounds in the development of new antimalaria treatments-an intro-duction. Malaria Journal. 2011;12(Supp 1): S2.
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