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Antimalarial Activity and cytotoxicity profile of the seed extracts of *Garcinia kola* (GUTTIFERAE)

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ABSTRACT

Background: The emergence of parasite resistance to artemisinins typified by slower parasite clearance rates in some parts of South east Asia has necessitated the search for alternatives. **Objective**: This study evaluated the *in vitro* and *in vivo* antimalarial activity and the cytotoxicity profile of the *n*- hexane, dichloromethane and methanol extracts of *Garcinia kola* seeds. **Methods:** *In vitro* susceptibility of chloroquine-sensitive PF D10 strain to the extracts was evaluated using parasite lactate dehydrogenase assay while cytotoxicity was determined using 3-[4,5-Dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide (MTT) assay with Vero cells and emetine as standard drug. Chloroquine-resistant *P. berghei* (ANKA) infected Swiss mice allotted into 14 groups of 5 per group: corn oil (5mL/kg), 50, 100, 200, 400 mg/kg of each extract and chloroquine (10 mg/kg) were used to evaluate *in vivo* antimalarial activity in a 4-day suppressive test. **Results**: The *n*-hexane, dichloromethane and methanol extracts of the seeds of *Garcinia kola* were active *in vitro* against chloroquine sensitive *P. falciparum* D10 strain with IC_{50} values ≤ 26 µg/mL. The hexane and dichloromethane extracts were non- cytotoxic against Vero cells with IC_{50} values ≥ 27 µg/ml The hexane extract of *Garcinia kola* seeds reduced parasitemia by 70% at 400 mg/kg and prolonged survival in mice infected with *P. berghei* ANKA. **Conclusion**: The observed antimalarial activity justifies the use of *Garcinia kola* seeds in the treatment of febrile illnesses.

Keywords: Antimalarial, *Garcinia kola seeds*, Cytotoxicity, Vero cells, Parasitemia.

INTRODUCTION

Malaria is an infectious disease affecting the tropical and subtropical regions of the world, including parts of the Americas, Asia and Africa, where it is endemic causing the death of hundreds of thousands of people annually especially children below the age of five and pregnant women [1]. The use of artemisinin-based combination therapy, insecticide treated nets and residual spraying in malaria endemic regions has yielded significant dividends resulting in a reduction in the morbidity and mortality attributable to the infection [2]. Unfortunately, the emergence and continuous spread of established artemisinin resistance across Southeast Asia mainland threatens global efforts to control and eliminate the most common and deadliest form of the malaria infection *Plasmodium falciparum* [3].

Medicinal plants have been in use for millennia and have been a valuable source of therapeutic agents, infact many of today's drugs are plant-derived natural products or their derivatives [4]. They have played a dominant and prominent role in the discovery of templates for the development of new drugs to treat diseases. Indeed, the existing antimalarial agents in use today are based on natural products and this could mean that new leads may likely emerge from tropical plant sources [5]. *Garcinia kola* Heckel *(Guttifereae)* seeds commonly known as bitter kola is a nut-bearing medium sized tropical forest tree found throughout West and Central Africa. *Garcinia kola* is mostly found in the southern part of Nigeria. It has a bitter taste followed by slight sweetness. The seed and leaves of *Garcinia kola* enjoys a folk reputation in Africa as a poison antidote [6] *Garcinia kola* is reported to possess hypoglycemic, antiparasitic, antimicrobial, antiviral, anti-inflammatory, antihepatotoxic and aphrodisiac properties [6, 7]. The seeds and leaf of the plant are used in folk medicine for the treatment of gram positive and gram negative bacteria, Ebola virus infections, dysentery and diarrhea [6, 7].

However, limited information on the *in vivo* antimalarial activity of seeds of *Garcinia kola* is available in literature. Efforts in this study were devoted to the evaluation of potential *in vitro* and *in vivo* antimalarial activity of the hexane, dichloromethane and methanol extracts *Garcinia kola* seeds against chloroquine-sensitive *P. falciparum* D10 strain and chloroquine- resistant *P. berghei* in mice, respectively. Furthermore, the cytotoxicity profile of the hexane, dichloromethane and methanol extracts *Garcinia kola*

was also evaluated using Vero cell line from the kidney of the African green monkey.

MATERIALS AND METHODS

Collection of plant materials

Samples of *Garcinia kola* seed (GKS) collected from farmers in Oyo town, Oyo state, Nigeria were identified and authenticated by plant taxonomists at Forestry Research Institute of Nigeria Ibadan with authentication number FHI 109896.

Preparation of Plant extracts

The seeds of *Garcinia kola* were air-dried and pulverised to a coarse powder using a table top blender. Five hundred grams (500 g) of the pulverised seeds was extracted exhaustively by cold maceration successively in *n*-hexane, dichloromethane and methanol at room temperature for 72 hours. The extracts were filtered, and the filtrates were concentrated to dryness using a rotary evaporator. The extracts were stored in a refrigerator (4°C) until required for assay.

In vitro antimalarial assay

Chloroquine-sensitive *P. falciparum* D10 strain derived from FCQ-27 from Papua New Guinea donated by the Division of Pharmacology at the University of Cape Town, South Africa was used for this study. The parasites were grown and maintained in continuous culture using a modification of the method of Trager and Jensen [8]. The culture medium consisted of 2% haematocrit suspension of O^+ human erythrocytes in RPMI (Roswell Park Memorial Institute Medium) 1640, supplemented with phenol red, 25mMNaHCO3, 25mMHEPES buffer (pH 7.4), and 50 μg/mL gentamycin and hypoxanthine. The reagents were obtained from Sigma-Aldrich, South Africa.

In vitro antimalarial activity was determined using the parasite lactate dehydrogenase assay [9]. The plant extracts (2 mg) were initially dissolved in DMSO (200 µL) and then diluted in complete culture media to 200 µg/mL. Two-fold serial dilutions were done with culture medium in a 96-well plate to give an extract concentration range of 0.195-100 µgmL. One hundred microliter (100uL) of an asynchronous culture of *P. falciparum* at 2% parasiteamia and 2% haematocrit was added to each well of the 96-well plate and incubated for 48 hours at 37 ºC. After incubation, the parasites were re-suspended by mixing gently using a multi-channel micropipette. Twenty microliter (20 µL) of the culture was taken from each well and added to a new 96-well microtitre plate containing 100 µL of the Malstat reagent in each well. Twenty-five microliter (25 µL) of a solution of 1.9 µM Nitro Blue Tetrazolium (NBT) and 0.24 µM Phenazine Ethosulphate (PES) were added to each well thus initiating the lactate dehydrogenase reaction.

Parasite growth was determined spectrophotometrically at 620nm with the aid of a microplate reader after an hour of incubation in a dark cupboard. Absorbance values obtained were expressed as a percentage of the 100% growth value and plotted against corresponding concentrations of the drug. The IC_{50} values of the plant extracts were obtained from dose-response curves, using non-linear dose-response curve fitting analyses with Graph Pad Prism version 5.0. Extracts with $IC_{50} \leq 10$ µg/mL were described as having good activity, while those with IC_{50} values > 10 µg/mL ≤ 30 µg/mL were described as having a moderate activity. Extracts with IC_{50} values ≥ 30 µg/mL were described as being inactive.

In vivo Antimalarial Tests

In vivo tests were performed according to the NIH guide for the care and use of laboratory animals, (publication number 85-23, revised 1985), and approved by the University of Ibadan ethical committee for the use of Laboratory animals. Inbred Swiss albino mice, weighing between 20-22g, aged 8-9 weeks, were used for all experiments. Animals were obtained from the animal house of the Malaria Research Laboratories, Institute for Advanced Medical Research and Training (IMRAT), University of Ibadan. The mice were housed in groups of five in plastic cages, fed with mouse cubes and provided with water ad libitum. The Peters' 4-day suppressive test was used for the *in vivo* drug tests [10].

Parasites

Chloroquine-resistant *Plasmodium berghei* (ANKA) strain used was obtained from the Malaria Research Laboratories, Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan. Parasitized red blood cells were obtained from donor-infected mouse by cardiac puncture in acid citrate dextrose (ACD) anticoagulant.

Animals

Seventy albino mice weighing 18-22 g were inoculated intravenously with 1×10^6 red blood cells infected with the CQ-resistant *P. berghei* ANKA strain. The infected animals were distributed randomly into 14 groups of 5 animals each and were treated with 50, 100, 200 or 400 mg/kg body weight of the hexane, dichloromethane and methanol extracts of GKS for 4 days. Two controls groups were used: one treated with chloroquine 10 mg/kg body weight given daily for 3 days while the second group of animals received corn oil (vehicle solution). Parasitemia was determined by microscopic examination of Giemsa–stained blood films obtained from the tail snips from each animal on day 4 after infection. The number of parasitized erythrocytes among 1000 RBC was counted and the percentage chemosuppression for each extract was calculated. Animal survival was monitored daily, until 30 days after infection.

1. Group I: Negative Control (received corn oil)
2. Group II: Positive Control (infected mice that received chloroquine 10 mg/kg body weight)
3. Group III-XIV: infected mice that received 50,100,200 or 400 mg/kg body weight of the hexane extract or dichloromethane extract or methanol extract of GKS

Cell viability assay

The Vero cells obtained from the kidney epithelial cells of the African green monkey were maintained in RPMI-1640 medium supplemented with 10% FBS, glutamine, penicillin (100 units/mL) and streptomycin (100 µg/mL). The cells were cultured at 37ºC in a humified 5% CO_2 incubator. The cytotoxicity of the seed extracts of *Garcinia kola* was evaluated against vero cells using the MTT (3-[4,5-Dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide) assay as described by Mossman [11] and emetine, was used as a standard cytotoxic agent. Briefly, the vero cells grown in standard tissue culture were plated at a density of $1x\ 10^6$ cells/mL/well (100 µL) into a 96-well plate. One hundred microliter (100 µL) of complete medium was also dispensed into the 96-well plate and cells were allowed to attach for 24 hours at 37°C. The medium was completely removed after 24 hours and replaced with

100 µL of fresh medium containing graded concentration of 100-0.0001µg/mL of the extracts of *Garcinia kola* seeds. A blank containing only complete medium served as a control containing no drug. The plates were then incubated at 37°C in 5% CO_2 for 48 hours. Thereafter, 25 µL of sterile MTT (5 mg/mL in PBS) was dispensed into each well and incubated for 4 hours at 37°C. Cell viability was measured at 540nm on a microtiter plate reader. Cell viability in the presence of the extract were compared to that of the negative control. The fifty percent inhibitory concentrations (IC_{50}) were calculated for the extracts and the positive control (emetine) using non-linear regression analysis. *In vitro* selectivity index was determined for each extract as the IC_{50} for Vero cells/ IC_{50} for *P.falciparum.*

Statistical analysis

Graph Pad prism version 5.0 (Graph-Pad Software, San Diego, CA, USA) was used for all statistical analyses. Fifty percent inhibitory concentration (IC_{50}) was calculated using non-linear regression analysis. Chemosuppression of parasite growth and survival of animals were expressed as mean ± SEM. Statistical significance of means of different variables were analysed using Student's t test and one-way analysis of variance between groups (ANOVA) was used to compare difference in percentage inhibition of parasite growth. For all statistical tests, $P<0.05$ was considered significant.

RESULTS

***In vitro* antimalarial activity**

The *in vitro* antimalarial activities of the three seed extracts of *Garcinia kola* against the D10 strain of *P.falciparum* is presented on Table 1. The dichloromethane extract exhibited the highest *in vitro* antimalarial activity, with an IC_{50} value of 10.59±0.64 µg/mL while the hexane and the methanol extract produced moderate *in vitro* antimalarial activity with IC_{50} values of 12.93±0.88 and 25.65±1.25 µg/mL against the D10 strain of *P.falciparum* respectively.

Table 1: Susceptibility profile of Chloroquine-sensitive *P. falciparum* D10 strain to seed extracts of *Garcinia kola*

Name of extract	IC_{50} (µg/mL)
Garcinia kola hexane	12.93 ±0.88
Garcinia kola dichloromethane	10.59±0.64
Garcinia kola methanol	25.65±1.25
Chloroquine	0.12±0.03

***In vivo* antimalarial activity**

The *in vivo* antimalarial activity of the hexane, dichloromethane and methanol extracts of GKS against *P. berghei* ANKA strain in Swiss albino mice is presented on Table 2. Mean parasitaemia on day 4 in the untreated control animals was 13.43±0.65%, while mean parasitaemia on day in animals treated with selected doses (50–400 mg/kg) of GKS extracts ranged from 4.5% to 16.9%. A dose-dependent chemosuppression of parasite growth was observed in animals treated with the hexane, dichloromethane and methanol extracts of GKS. The hexane extract of GKS produced the highest chemosuppression of 69.9% at a dose of 400 mg/kg, while Chloroquine completely suppressed parasitaemia on D4. At 400 mg/kg of the hexane extract of GKS tested, there was a significant percentage inhibition of parasiteamia compared to the untreated control group ($P \leq 0.05$), with the longest survival time of 15 days. All the doses of the dichloromethane and methanol extracts of GKS tested did not produce significant chemosuppression of parasiteamia. However, 400 mg/kg of the dichloromethane extract of GKS, produced a mild chemosuppression of parasitaemia of 47.95%. It was also interesting to note that animals in that were treated with selected doses of the dichloromethane and methanol extract survived longer than the animals that were untreated.

Table 2: *In vivo* antimalarial activity of the seed extracts of *Garcinia kola* against chloroquine resistant *P.berghei* in Swiss albino mice

Dose mg/kg	Parasiteamia ± SEM (%)	Chemosuppression ± SEM (%)	Survival time (days)
GK HEX			
50	13.55±0.35	0.00±0.00	11.75±0.25
100	9.07±2.46	33.80±3.70	12.20±0.20
200	7.23±1.78	47.00±1.00	13.35±0.25
400	4.46±1.17	69.89±9.79	14.60±0.20
GK DCM			
50	11.12±0.24	17.50±2.00	9.70±0.70
100	8.69±0.93	35.50±7.50	12.65±1.15
200	8.83±0.79	34.30±6.29	13.10±2.20
400	7.00±0.35	47.95±2.75	16.20±1.20
GK MEOH			
50	16.91±0.79	0.00±0.00	9.70±0.50
100	12.11±2.85	9.90±0.30	12.50±0.10
200	10.68±2.61	21.05±0.75	13.50±0.30
400	8.74±2.45	36.35±4.25	14.60±0.20
C/Q 10mg/kg	0.00±0.00	100.00±0.00	31.50±3.50
Corn oil	13.43±0.65	0.00±0.00	10.60±0.20

CQ = Chloroquine diphosphate (10 mg/kg/day), Corn oil (Untreated control)
GK HEX= *Garcinia kola* hexane extract
GK DCM= *Garcinia kola* dichloromethane extract
GK MEOH = *Garcinia kola* methanol extract

Cytotoxicity activity and Selective indexes of the seed extract of *Garcinia kola*

The Cytotoxic activity of the hexane, dichloromethane and methanol extracts of *Garcinia kola* seeds against vero cells are shown in Table 3. The IC_{50} values of the extracts against vero cells ranged from 7.00 µg/mL to 76.78 µg/mL. The hexane extract of *Garcinia kola* seeds was the least toxic (IC_{50} = 76.78 ± 2.00 µg/mL) while the methanol extract was the most toxic (IC_{50} = 7.00 ± 0.52 µg/mL). The IC_{50} value of emetine, a standard cytotoxic agent which served as the positive control was 8.00 ± 0.60 µg/mL. High selectivity indices indicate a strong selective ability of an agent to destroy the malaria parasite. Selectivity index of the extracts of *Garcinia kola* seeds ranged between 0.27 and 5.94 (Table 3).

Table 3: *In vitro* cytotoxicity profile of the seed extracts of *Garcinia kola* against Vero cells

Extract	IC_{50} (µg/mL)	Selectivity index
Garcinia kola hexane	76.78 ±2.00	5.94
Garcinia kola dichloromethane	27.99±1.96	2.64
Garcinia kola methanol	7.00±0.52	0.27
Emetine	8.00±0.60	N/A

Selectivity index = IC_{50} (Vero) / IC_{50} (D10), N/A = Not applicable

DISCUSSION

In the absence of an efficient and readily available vaccine for the prevention of malaria, chemotherapy still remains the main stay for the treatment of malaria. The emergence and spread of Artemisinin resistant parasites across Southeast Asia is a major setback in the progress made on a global basis in malaria chemotherapy [12]. One possible source for a replacement of the artemisinins might lie in nature based on the antecedence of antimalarial drug discovery. Ethno medicine has produced two major antimalarial agents: Quinine the drug of choice for the treatment of malaria for several decades was sourced from plants called *Cinchona calisaya* and *Cinchona Succirubra* and Artemisinin from the plant called *Artemisia annua.* [4, 13]. This has necessitated the search for newer and more potent antimalarials from plant sources. In this study, efforts were directed at evaluating the antimalarial potential and the cytotoxicity profile of *Garcinia kola* seeds.

Garcinia kola is an angiosperm belonging to the family Guttifereae/Clusiaceae. It is a highly valued ingredient in African ethno medicine because of its varied uses which include medicinal and social uses. Results from this study indicate that the hexane, dichloromethane and methanol extracts of *Garcinia kola* seeds possess good to moderate *in vitro* antimalarial activity, however, only the hexane extract of *Garcinia kola* seeds demonstrated the highest activity *in vivo* and was against the asexual erythrocytic stages of *P. berghei.* The exact reason for this is not fully understood however, various factors such as first pass effect, poor absorption and route of administration of the extracts may have influenced the observed activity of the extract. The hexane extract of *Garcinia kola* seeds was observed to show good intrinsic *in vitro* and *in vivo* antimalarial activity judging by the inhibitory concentration value obtained and its percentage chemosuppression *in vivo* in comparison with that of chloroquine, although chloroquine had a better activity in *vitro* and *in vivo,* which could be due chloroquine's purity and the extracts crude nature.

It is interesting to note that the hexane and dichloromethane extracts of *Garcinia kola* seeds were found to be non-cytotoxic to normal monkey epithelial cells. The ability of the hexane extract of *Garcinia kola* seeds to destroy *P. berghei* parasites in Swiss albino mice and cause no harm to the vero cells, which are normal epithelial cells of the African green monkey is an its indicator of selective toxicity, and as such can be considered as potential lead for clinical management of malaria. In contrast, the cytotoxicity of the methanol extract of *Garcinia kola* seeds was comparable to emetine, the standard cytotoxic drug used. This finding suggests that the methanol extract of *Garcinia kola* seeds may have potentially useful anticancer properties.

CONCLUSION

The hexane extract of *Garcinia kola* seeds showed *in vitro* and *in vivo* antimalarial activity and was non-cytotoxic to vero cells, justifying its use in folklore for the treatment of febrile illnesses. There is however a need to purify the hexane extract of *Garcinia kola* seeds in order to identify the compound (s) responsible for the observed antimalarial activity, which could serve as templates or leads for the development of new alternatives to the artemisinins.

Conflict of Interest

The authors declare that there is no conflict of interest as regards this article.

REFERENCES

1. World Health Organization, World malaria report 2018. Geneva.
2. Woodrow CJ, White NJ. The clinical impact of artemisinin resistance in Southeast Asia and the potential for future spread. FEMS Microbiology Reviews. 2016; 41(1):34-48.
3. Fairhurst RM, Dondorp AM. Artemisinin-resistant *Plasmodium falciparum* malaria. Microbiology Spectrum. 2016; 4(3):10-13.
4. Kingston DGI, Cassera MB, Su Q, Du Y, Presley C, Dalal S, Goetz M. Discovery and biology of novel antimalarial agents from plants. Planta Medica. 2016; 82:S01-SL31.
5. Buba CI, Okhale SE, Muazzam I. Garcinia kola: The phytochemistry, pharmacology and therapeutic applications. International Journal of Pharmacognosy. 2016; 3(2):67-81.
6. Ogbadoyi EO, Kabiru AY, Omotosho RF. Preliminary studies of the antitrypanosomal activity of *Garcinia kola* nut extract in mice infected with *Trypanosoma brucei* brucei. Journal of Medicine and Medical Sciences. 2011; 21: 628-631.
7. Okhale SE, Buba CI, Oladosu P, Egharevba HO, Ugbabe G, *et al.* Chemical constituents and antimicrobial activity of the leaf essential oil of *Garcinia kola* Heckel (Clusiaceae) from Nigeria. American Chemical Science Journal. 2016; 13(4):1-7.
8. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science. 1976; 193(4254):673-675.
9. Makler MT, Hinrichs DJ. Measurement of the lactate dehydrogenase activity of *Plasmodium falciparum as an* assessment of parasitemia. American Journal of Tropical Medicine and Hygiene. 1993; 48:205-210.
10. Peters W, Portus JH, Robinson BL. The chemotherapy of rodent malaria, XXII: the value of drug-resistant strains of *P.berghei* in screening for blood schizontocidal activity. Annals of Tropical Medicine and Parasitology. 1975; 69(2):155-171.
11. Mossman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods. 1983; 65(1-2):55-63.
12. Blasco B, Leroy D, Fidock DA. Antimalarial drug resistance: linking *Plasmodium falciparum* parasite biology to the clinic. Nature Medicine. 2017; 23(8):917-928.
13. Klayman DL, Lin AJ, Acton N, Scovill JP, *et al*. Isolation of artemisinin (qinghaosu) from *Artemisia annua* growing in the United States. Journal of Natural Products. 1984; 47(4):715-717.

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