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Research Paper

Antiplasmodial *Potentials* of Ethanol extract of *Garcinia Kola* (Bitter kola)

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ABSTRACT: *This study was aimed at determining the in vitro antiplasmodial activity of ethanol extract Garcinia kola (Bitter kola) on Plasmodium falciparum. The inhibitory effects of aqueous and ethanolic extracts of G. kola on P. falciparum were determined at five different concentrations (100, 50, 25, 12.5 µg/ml). The results obtained from in vitro antimalarial activity shows that the highest inhibition rate was recorded at 100µg/ml concentration, while the least was recorded at 12.5µg/ml, thus, it can be hypothesized that the activity of the extracts on the parasite is dose dependent. In conclusion, this present research has confirmed that G. kola extracts contains potent antimalarial agents. Therefore, it is recommended that further studies should be conducted using higher concentrations of the extracts at molecular levels for a more positive outcome and enhanced therapeutic modalities of administration.*

KEY WORDS: *Garcinia kola, Plasmodium Falciparum, Inhibition, Antimalaria*

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I. INTRODUCTION

Plasmodium is a parasite which infects the red blood cells and causes the disease known as Malaria. Of the species known to infect man, *Plasmodium falciparum* is the most deadly and predominant in Africa [1]. Malaria is a tropical disease of public health importance endemic in Africa, Asia and parts of America. Recent data revealed that globally, there was an estimated 229 million cases of malaria in 2019, which lead to 409,000 deaths. Africa alone accounted for about 219 million of the global cases, recording for about (95%) of all deaths worldwide. Nigeria alone is responsible for highest prevalence (27%) and of mortality rate (23%) globally [2]. Mortality and morbidity caused by Malaria seriously affects productivity and economic development. Direct costs through illness, treatment and premature death have been estimated to be at least US$ 12 billion per annum globally [3]. It accounts for about 60% of out-patient hospital admission, 30% childhood deaths and 25% of deaths in infants less than 1year in Nigeria [4]. Malaria has been a serious socioeconomic problem requiring an estimated US$ 4.5 billion to implement the National Malaria Strategic Plan (NMSP) 2014-2020.

There is a major concern about the development of resistant strains of *P. falciparum* to most of the antimalarial drugs that are currently in use. This resistance has been attributed to factors such as production of substandard drugs and incomplete dose. The cost of qualitative antimalarial drugs in developing countries is discouraging, thus; patients seek other traditional alternatives from plant materials [5].

Garcinia kola (Bitter kola or Heckel in English, *Namijingoro* in Hausa, *Orogbo* in Yoruba, *Aka ilu* in Igbo), belongs to the family Guttiferea.It is a perennial crop growing in the forest, distributed throughout West and Central Africa [6]. *G. kola* is also found distributed in the forest zone of Sierra Leone, Ghana, Cameroon and other West African countries. In Nigeria, it is common in the South Western States and Edo State [7]. Most of them remain in wild and semi-domesticated forms of regional importance but have been re-discovered as so-called neglected or underutilized crops [8]. The leaves are simple, 6-14 cm long and 2-6 cm across, shiny on both surfaces and spotted with resin glands. The small flowers are covered with short, red hairs [6]. *Garcina kola* fruit is a drupe of 5-10 cm in diameter and weight between 30 to 50 g. The fruit changes colour during maturation from green to orange, and each fruit contains 1-4 smooth elliptically shaped seeds [9].

Extracts of the plant have been used traditionally for treatment of Lanryngitis, cough and other uses [10]. Positive results from similar studies on the antiplasmodial potentials of *G. kola*, have generated a lot of enthusiasm about its importance in the control of Malaria. Although, the continous discovery of new variant

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strains of the most deadly resistant *P. falciparum* species has been and still a huge setback the search for effective malaria drugs. This research is aimed at validating the efficacy of *G. kola* extracts on 3D strain of *P. falciparum*, in a bid develop a more affordable and potent antimalarial drug that will be readily available to endemic African communities in the quest to control the disease, considering the plant's abundant existence in the region.

II. METHODOLOGY

2.1 Collection of plant materials

The *Garcinia kola* plant also known as "Na Mijin-Goro" was purchased from Mubi main Market in Adamawa State, Nigeria and taken to an experienced taxonomist at the Department of Plant Sciences, Adamawa State University Mubi, Nigeria, for proper identification.

2.2 Extraction of plant material

The wholesome seeds were peeled manually to remove the brown seed coat. *G. kola* seeds was thoroughly washed with distilled water and then cut into small bits and placed on a sterile laboratory tray for fast sun drying. The seeds were crushed to coarse powder using a neatly washed local mortar and pestle. The milled samples were packaged in sterile screw capped sample bottles and stored at ambient temperature for [11].

2.2 Preparation of Sample

Ethanol was used as solvent, for extraction of active compounds in the plant seed *(G. kola)*. 50g of processed plant seed was soaked in 150 ml of 90% ethanol in 250 ml conical flask and then covered with aluminum foil paper. This was shaken vigorously twice in a day and allowed to stand for 48 hours to effect proper extraction of active ingredient. The suspension was filtered with Whitman's filter paper to obtain the supernatant while the debris was discarded.

2.4 Culturing of *Plasmodium falciparum* parasite

The 3D7 strain of *P. falciparum* was obtained from National Veterinary Research Institute VOM, Plateau State. The Parasites was kept in culture with complete parasite medium, CPM (RPMI 1640 supplemented with 10% Albumax II, 50 µg mL–1 gentamycin, 1% L-glutamine). Parasites were cultured with O+ RBCs at 4% haematocrit. For periodic maintenance, parasitaemia was checked by Giemsa staining and the culture replenished by addition of an appropriate amount of fresh O+ RBCs, flush with a gentle flow of mixed gas (5.5% CO2, 2.5% O2 in N2) for at least 30 sec and kept in a 37°C incubator [12].

2.5 Sorbitol synchronization of the parasites

Sorbitol treatment was used to enrich the ring stages of the parasite for assay purposes and was performed when most of the parasites are at the ring stage. The culture was transferred into a 15ml falcon tube and centrifuged for 10 min at 350×g after which the medium (supernatant) was removed. To the pellet, was added 5 mL of a 5% sorbitol solution with gentle shaking and the mixture allowed to stand for 10 min at 37°C. The mixture was subsequently spun at 350×g for 10 min and the supernatant removed. The pellet was then washed twice with parasite wash medium (RPMI 1640 with 50 µg mL–1 gentamycin, 1% L-glutamine) and finally with CPM. A thin smear was later prepared to check for the effectiveness of the synchronization procedure. The pellet was used to inoculate a T-25 culture flask at 4% haematocrit, gassed for about 30 sec and kept in an incubator at 37°C for at least one schizogonic cycle before use in an assay.

2.6 *Invitro*antiplasmodial assay

A stock solution of 100 mg mL^{-1} of the ethanol extract was prepared in Phosphate Buffered Saline (PBS) and was sterilized by filtration through a 0.22 µm membrane (Millipore). The Filtrate assay was serially diluted to 1000µg ml^{-1} in CPM and this was serially diluted to finally yield four concentration of extracts for the assays (100, 50, 25 and 12.5µg ml^{-1}). These dilutions were added in triplicates to a 48-well tissue culture plate containing 3D7 strain *P. falciparum* ring stages of 0.7 % parasitaemia and at 3% haematocrit. The culture medium was used as negative control, while positive control was made with Chloroquine-Diphosphate. After 24, 48 and 72 hours of incubation at 37ºC the contents of the wells were harvested and a thin blood film was prepared for parasitesmia estimation. The film was fixed with methanol, stained for 10 minutes in 10% Giemsa and viewed under a light microscope after washing and drying. The parasite count was recorded and the percentage parasites inhibition was calculated using the formula;

$$\text{Inhibition}(\%) = \frac{\text{mean control parasitesmia} - \text{mean test parasitesmia}}{\text{Mean control parasitemia}} \times 100$$

III. RESULTS

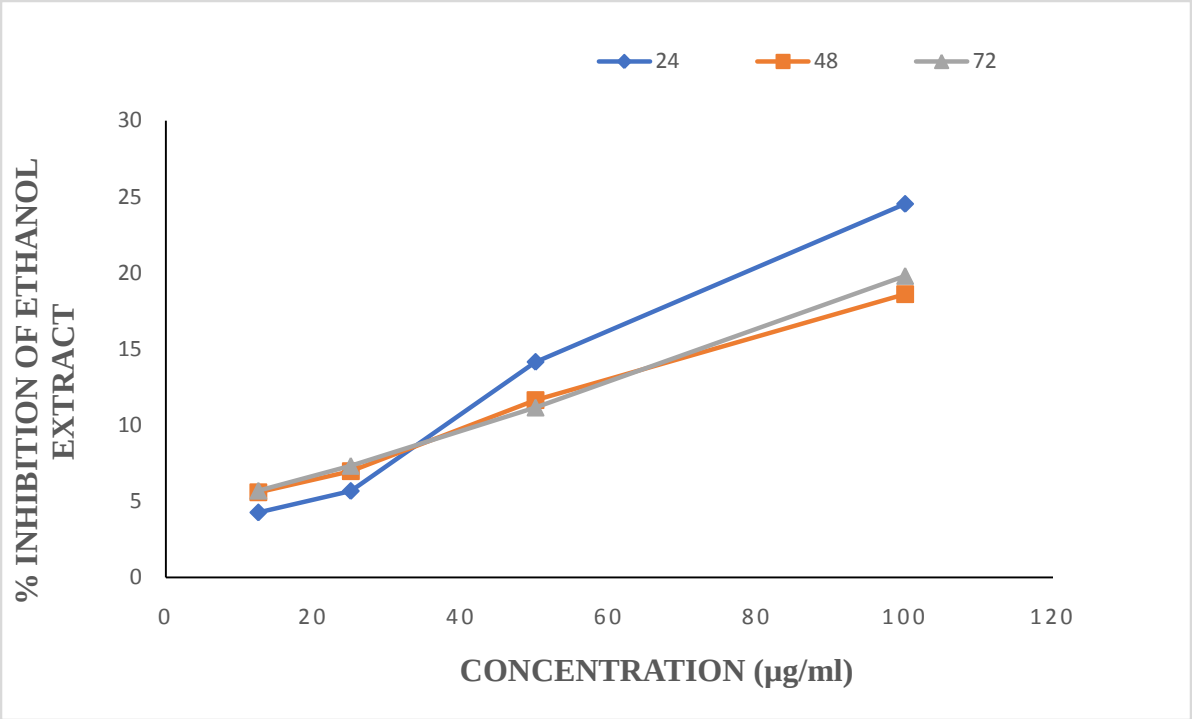

Figure 1: Percentage inhibition of ethanol extract of *G. kola* on *plasmodium falciparum*

Table 1: *In vitro* antimalarial activity of ethanol extract of *G. Kola* (Bitter Kola) on *P. falciparum*

Concentration of Extract (µg/ml)	Time (Hours) 24	48	72
100	53.33±2.33^{c}	58.33±0.66^{c}	58.00±1.00^{c}
50	60.66±1.20^{c}	63.33±3.17$^{a.}$	59.00±5.19^{a}
25	66.67±0.88^{a}	66.66±3.84$^{a.}$	67.00±2.08^{c}
12.5	67.67±1.85^{a}	69.00±3.46^{a}	69.67±0.88^{a}
-ve control	70.67±1.20^{a}	71.67±0.88^{a}	72.33±0.88^{a}
+control (CQ) 50µg/ml	3.33±0.88^{b}	4.67±1.20^{c}	8.33±1.45^{a}

Values are mean ± SD. Values with the same superscript letters across the rows are statistically not significant from each other ($p<0.05$).

IV. DISCUSSION

Malaria is the deadliest parasitic disease globally, with Africa accounting for about 95% of cases and death. Till date there is no substantive date on when an effective vaccine will be available for this killer disease (malaria), because the world health organization has proposed ending 2021 before it will start review of evaluation of data from pilots together with the results of several studies conducted since 2015, for consideration and advisability of broader use of the vaccine. As the world waits for the outcome of an effective vaccine, the emergence of new artemisinin resistant strains of *P. falciparum* malaria parasite endemic across sub-saharan Africa is a major setback in the progress made on a global basis in malaria chemotherapy [13]. These challenges have made it necessary to seek for newer alternatives aside from conventional drugs of choice. It is a well-known fact that Ethno-medicine has produced the two major antimalarial agents: Quinine the drug of choice for decades was sourced from the Genus Chinchona and Artemisinin from Artemisia annua [14], thus, serving as a motivation in the quest for affordable, available and more potent antimalarial drugs from other plant sources. This study was conducted to evaluate and validate the potential of ethanol extract of *G. kola* on the development of 3D strain of *P. falciparumin vitro*. The antimalarial activities of *G. kola*seeds as summarized in Table 1 revealed that the plant seed extracts have potentials of inhibiting the growth and development of *P. falciparum*, which is similar with the findings of [15, 16, and 17].It is of noteworthy that antimalarial activity of the extract increased as the concentrations increases, which probably mean that the efficacy is dose dependant. Interestingly, it was observed in this present study that the effectiveness of the treatments on the level of parasitemia slightly reduced over time (24hrs>48hrs>72hrs respectively). Plasmodium parasites have been reported to evade immunity through intracellular survival and creation of rosettes, thus, avoiding direct contact and recognition by immune cells respectively [18]. Therefore, there is that tendency of the *P.*

*falciparum*parasites exhibiting similar behavior on exposure to active antimalarial components of *G. kola* in order to enhance their chances of survival and further development over time.

V. CONCLUSION

Results from this present research have proved that *G. kola* extracts contains potent antimalarial agents that can inhibit the development of *P. falciparum*. It is therefore recommended that further studies should be conducted using higher concentrations at molecular levels for a more positive outcome and enhancement of therapeutic modalities of administration.

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