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Exploring the *in-vitro* and *in-vivo* trapanosomal Activities of *Gacinia kola* (Bitter kola) Seed Aqueous Extract using Animal Models

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Abstract
Trypanosomiasis (sleeping sickness) is a significant disease of economic and public health importance caused by Trypanosoma brucei gambianse parasite that affects humans. In 2014, WHO stated that 300,000 cases were reported to be diagnose and treated, and others died or lived with a disability based on the non-availability of the novel drugs, high toxicity, and development of resistance to the drug by the parasite. Therefore, using plant extract is fast becoming the choice for treating the disease. This study investigated the in-vitro and in-vivo trypanosomal activities of Garcinia kola (Bitter kola) aqueous seed extract as an alternative replacement for Diamininzene aceturate drug in the treatment of Trypanosomiasis Disease using animal modeling. The extract was obtained through the Maceration method with distilled water, and photochemical analysis was performed using various standard methods. In-vitro activity of the extract and Diaminizene aceturate drug was determined using rapid matching techniques at 3 hours post incubation with different doses of the extract and 3.5 mg/kg/bw of standarrd drug. In-vivo study was conducted using sixty-two (62) Wister rats divided into ten groups. Group A (standard control): noninfected and non-treated but received 10mL of distilled water; Group (B-H) were infected by intraperitoneal inoculation of 1mL of parasitized blood containing 1.0×10^5/ml of T. brucei gambianse parasites and treated with different doses of Garcinia kola seed extract, 200, 400 – 1400 mg/kg/bw; Group I: infected and treated with standard drug dose (Standard control); Group J: infected and untreated (Pathological control). The treatment lasted for 21 days at 3-day intervals. Data were analyzed statistically using Anova Turkeys post hoc SPSS version 2.0 software, with a significant difference at 0.01. The result revealed the photochemical components of the seed as Flavonoids, Steroids, tannins, saponins, Anthraquinones, glycosides, and carbohydrates. Garcinia kola seed extract exhibited trypanosostatic at doses of <400 mg and trypanocidal activity at doses > 400 mg in both in vitro and in v and in vivo, compared with trypanocidal activity of standard drug dose at 3.5 mg/kg/bw. Garcinia kola aqueous seed extract possesses trypanosostatic and trypanocidal effects in both In vivo and In vitro. Diamininzene aceturate drug proved to be trypanocidal at standard recommended dose. This study revealed that Garcinia kola aqueous seed extract might be used to design an alternative drug for treating trypanosomiasis disease at a lower concentration of 200mg/kg/BW.

Keywords: *Trypanosomiasis, Gacinia kola, Trypanocidal, Berenil drug, in-vivo and in-vitro*

INTRODUCTION
Sleeping sickness is caused by the subspecies trypanosome, *Trypanosoma brucei brucei gambiense,* and *T. brucei rhodesiensie*. This parasite lives and multiplies extracellular in their human host's blood and tissue fluid and is transmitted through the bite of infected tsetse flies (*Glossina* spp) (Barett *et al*., 2002). The occurrence of this disease is restricted to the distribution of tsetse fly, which is exclusively found in sub-Sahara Africa between 14°N and 20°N (Barett *et al*., 2002). More than 250 discreet active trypanosomiasis disease foci in 36 African countries are mostly recognized many of which are in rural areas (WHO, 2005). *Trypanosoma brucie rhodesiense* is mainly found in East and South Africa where as *T.b gambiense* occur in Central Africa and West Africa countries. The course of trypanosomiasis differs depending on the subspecies; infection with *T. b rhodesiense* leads to a moderate form of the disease, while infection with *T.b gambiense* gives rise to a chronic infection (WHO, 2005). The pathological symptoms of the first stage of the sickness, defined by the restriction of trypanosome to the peripheral blood and lymphatic systems, include fever, headache, gut pain, and itching (Abubarkar, 2012; WHO, 2005). The clinical sign of trypanosomiasis's second stage is characterized by trypanosome parasites in the central nervous, neurological, and endocrinal systems. If left untreated, trypanosomiasis patients infected with T.b rhodesense might die within months. In contrast, those infected with *T. brucie gambiense* may survive for several years due less to the pathological effects of the parasite (Emmanuel *et al.,* 2011).

In the late 19th century, Africa experienced a severe sleeping sickness epidemic, the most worrisome of which was an epidemic with 300,000 to 500,000 deaths of patients between 1896 and 1906 that mainly affected the Congo River Basin and the Busoga foci point in Uganda and Kenya (Stake, 2015; WHO, 2005). The devastating effect of this epidemic motivated the various colonial

administrations to call for their medical scientists and experts to develop a cure for trypanosomiasis disease. At that time, the era of chemotherapy was developed and began to make use of the novel method of medical chemistry via the identification, synthesis, and development of new chemical entities suitable for therapeutic use and its effect, which led to the production of early anti sleeping sickness drug that medical chemist was first used (Mameri et.*al*., 2004).

Garcinia kola, locally known as Orogbo in Yoruba," Namijin" goro in Hausa, and Akilu in Igbo languages of Nigeria, and commonly called bitter kola in English, belong to a class of plants described as masticators (Adeyemi *et al*., 2012). It is found mainly in the forest region and grows as a medium-sized tree, up to 12 m in height. The plant is cultivated and distributed widely throughout West and Central Africa, where it is valued for its edible nuts. The hard nut is chewed to realize its bitter content, traditionally believed to be a stimulant of the central nervous system and an enhancer of male potency. Medicinal uses *of G. kola,* as reported in the literature, include its use as an antiseptic, antimicrobial, antiviral, anti-inflammatory, purgative, and antidote to the effects of *Strophanthus gratus*, for guinea worm infection and also a remedy for the treatment of gastroenteritis, rheumatism, asthma, menstrual cramp, bronchitis, throat infection, head or chest cold, cough, and liver disorder (Lutje *et al*., 2013). The plant seed is also an antidiabetic and antioxidant for the chemo-prevention of aflatoxin B and anti-hepatotoxic activity. The phytochemicals obtained from *G. kola* as documented in literature including, Biflavounoids, Xanthone, kola-none, ameakoflavour, 24 methylene cycloartenol, coumarin and prenylated benzophenone (Madubuyi, 2003). Diamininzene aceturate is an aromatic diamidine produced by Hoechst to treat bovine trypanosomiasis. However, its apparent low incidence of adverse reactions and significant therapeutic effect has led some physicians in endemic countries to use it extensively for human sleeping sickness. It is effective against the early stage of *T.b. gambienses* and *T. b. rhodesenses*. This drug has also been used in combination with Melarsoprol for the last stage of the disease. Like pentamidine, Berenil can be linked to kinetoplast DNA binding at the minor groove and cleavage of minicircle Deoxyribonucleic acid. Although with pentamidine, this drug may also interfere with Ribonucleic acid editing and transplanting, It is also an effective and noncompetitive inhibitor of Adomet decarboxylase in trypanosomes, resulting in the reduction of spermidine content and elevating putrescine in the parasites. Based on the fact that the disease affected mainly the rural poor people and is more likely to affect public health, investment, and development of international drugs. In 1995, a WHO expert committee estimated that 60 million people were at risk of trypanosomiasis disease, 300,000 cases per year in Africa, and fewer than 30,000 cases diagnosed and treated (WHO, 2014).

In 2004, the number of new cases reported dropped below 10,000 (9987) for the first time in 50 years, and the estimated number of actual cases was 30,000 (WHO, 2009). This plummeting trend continued, and in 2014, only 3796 cases were reported, with less than 15,000 estimated cases (Kennedy, 2013).

Drugs for the treatment of the disease are no longer available in the market, and it is significant that since 1985, only novel types of trypanocidal drugs have been produced and only for use against trypanosomes in men (Franco *et al*., 2014). For the centuries of the African bovine *trypanosomes,* the only drugs currently used are Diminazene aceturate, homidim salt, and isometamidium chloride (Samorin) and Melasorprol. All have been employed in treatment for 30 years or more; therefore, we will need to consider the use of tradomedical medicine and the exploitation of the medicinal properties of some local plants for the combat of the menace of this disease both on man and animal, since in recent time, it has been estimated that 35 million people die of trypanosomiasis yearly throughout African countries (WHO, 2009). Therefore, this study investigated the In-vitro and in vivo anti trypanosomal activities of Garcinia kola (Bitter kola) aqueous seed extract as an alternative replacement of Diamininzene aceturate drug in the treatment of Trypanosomiasis Disease using animal modeling**.**

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Garcinia kola (Bitter kola) seed was purchased from Relief Market in Owerri Municipal in Imo State, Nigeria, identified and authenticated by a Botanist in the Department of Biological Sciences of Chukwuemeka Odumegwu Ojukwu University, Uli without voucher specimen number.

Plant Preparation

Eight hundred (800) pieces of the plant seed were prepared by peeling the coating and chopping the seed into small pieces to allow easy drying at normal room temperature. The dried pieces were ground into powder form using a mortar and pestle and stored in a plastic bottle until required for use.

Drug Source

The Diaminiezine Acurate (Diaminizene aceturate) was produced by Hebel ChangSheng TangAnimal pharmaceutical Co .LTD and imported from India through Biotan Hong Kong Co, Limited and identified and confirmed by a Pharmacist from Federal Polytechnic Nekede Owerri Imo State Nigeria.

Trypanosome

Trypanosome brucei gambiense was obtained from stability maintained at the Nigeria Institute of Trypanosomiasis and Onchocerciasis Research and Control Vom Plateau State, Nigeria. Afterward, it was maintained in the Biological Science Laboratory of COOU Uli by continuous passage of infected blood into healthy rats.

Animals

Albino Wister rats weighing 152 – 250 grams were purchased from the Pharmaceutical Technology Department of Federal Polytechnic Nekede Owerri Imo State, Nigeria. The experiment was conducted following the Canadian Council on Animal Care's (CCAC, 1997) Guideline on Animal Used Protocol Review.

Preparation of Aqueous Extract of the Plant Material

250 grams of dried powdered *Garcinia kola* seed was dissolved in three liters of distilled water and stirred vigorously at intervals of 1 hour for four hours, and it was allowed to stand on a bench for an hour without disturbance. The solution was later refrigerated for twenty-four hours and sieved with a laboratory sieve of 0.5 µ sieves and let to stand for one hour to allow its heavy particles to settle down. The supernatant was decanted and filtered using Whatman filter paper. The residue was transferred into an open tray, dried in the oven at 100 °C for 2 days, scraped with a spatula, and ground into a fine powder using a laboratory pestle and mortar (Igboli *et al*., 2011). Percentage yield was calculated using the formula.

$$\%\ yield = \frac{weight\ of\ extract}{weight\ of\ powdered\ seed} \times \frac{100}{1} \qquad (3.1)$$

Acclimatization of Animals

Sixty-six (66) Wistar rats were housed in a well-ventilated, clean iron cage with standard housing conditions. Temperature of 28–37 °C, photoperiod of 12 hours, and humidity of 50–55 % were used for this study. The animals were allowed free access to poultry feed and tap water; the cage was cleaned daily. The animal was acclimatized for 4 weeks before the commencement of the study. The Wister rats were randomized entirely into ten groups: A, B, C, D, E, F, G, H, I, and J, comprising six animals each and four (4) animals for acute toxicity study in separate cages (Atawodi, 1998).

Infection of Animal

Blood from a highly parasitized rat from the Nigeria Institute of Trypanosomiasis and Onchocerciasis Research and Control Vom Plateau State, Nigeria, anesthetization was obtained from cardiac puncture using a syringe and needle. The blood was collected into a blood specimen collection bottle containing ethylene-diamine tetra acetic acid (EDTA) and diluted with dextrose saline solution using a serial dilution method up to 10^5 to serve as inoculums. Healthy rats were inoculated intraperitoneal with 1.0 mL of the inoculums, which contain about 1.0× 105 trypanosome/ml (Emmanuel *et al*., 2011).

Study Design

Ten (10) groups consisting of six (6) healthy albino rats were set up; Group A was not infected, well fed, and treated with 10 mL of distilled water to serve as standard control; Group B, C, D, E, F, G and H were infected and treated with different graded doses of *Garcinia kola* seed aqueous extract, 200, 400, 600, 800, 1000, 1200 and 1400 mg per kilogram body weight at 3 days intervals of post-infection, Group I was infected and treated with 3.5 mg/kg/bw of standard Berenil drug to serve as standard control, Group J was infected and untreated to serve as pathological control).

The test and control groups' treatment lasted 21 consecutive days, with the plant seed extract administered orally and the standard drug administered intramuscularly. Afterward, the in vitro and in vivo trypanosomal activity of Garcinia kola aqueous seed extract and Berenil drug was determined using standard methods.

Phytochemical Screening of* Garcinia kola *Seed Aqueous Extract

The method of Evans (1996) was used for most Phytochemical screening, while Omwirhimen *et al*. (2010) method was used to screen for only Terpenes and Quinines.

Determination of Oral Toxicity Limit test dose of the Extract

The oral toxicity limit test dose of the extract was determined using a test limit dose of 2000 mg/kg/bw according to the Organization of Economic Co-operation Development (OECD) protocol for testing chemicals using Rats or Mice (OECD, 2001). Four albino rats separately received orally 2000 mg/kg/bw of

the *Garcinia kola* seed aqueous extract. The rats were monitored continuously for 1 hour after administration of the extract intermittently for 4 hours over 24 hours for 10 days for gross behavioral changes and other signs of toxicity manifestation. The acute toxicity study revealed that there were no significant signs of acute toxicity, and death was not observed at the limit dose test of 2000 mg during the 10-day observation period of the animals.

Determination of Trypanosomal Activity

***In-vitro* Study of Aqueous Extract of Garcinia Kola Seed and Berenil Drug on *Trypanosoma brucei gambiase* Parasite**

Forty (40) test tubes were used. The test tubes were sub-divided into groups (A – H) of five test tubes each. Cardiac blood collected from one of the donor rats at the peak of parasitemia (250×10^6/mL) was diluted serially with 10ml of dextrose saline. The aliquots of 0.5 mL containing 5.0×10^5/ml of the parasite were pipetted into each test tube for Group (A – H). Test tube A was treated with 200 mg/mL, Group B with 400 mg/mL, Group C with 600 mg/mL, Group D with 800 mg/mL, Group E with 1000 mg/mL, Group F with 1200 mg/mL, Group G with 1400 mg/mL of the extract and Group H with 3.5 mg/kg of Berenil drug per 12.0 mL of distilled water. All the test tubes were incubated at 37 ^{o}C room temperature. The level of parasitemia in the test in each tube was determined at 3 hours intervals for 24 hours using the rapid matching technique of Herbert and Lumsden (1976) to count the number of parasites per field under the light microscope at x40 since motility constitutes a relatively reliable indicator of viability among most zooflagenate parasite (Peter *et al*., 1976). Cessation or drop in the parasite's motility was used to evaluate the anti-trypanosomal effect of the plant extract and standard drug under *in-vitro* conditions.

***In-vivo* Study of Aqueous Extract of *Garcinia Kola* Seed and** standard **Drug on *Trypanosoma brucei gambiase* Parasite**

Animals were observed for daily death, and a tail vein blood smear was performed at a 3-day interval after post-infection treatment to determine the parasite load and the effectiveness of the treatment based on the dosage of both extract and the standard drug.

METHOD

Blood (20 µm) was placed on a greased-free slide, then covered with a cover slip, and examined under a microscope with x10 and x40 objective lenses for motile trypanosomes. A direct smear count was carried out using Herbert and Lumsden's standard rapid matching counting technique of 1976.

Statistical Analysis

The data obtained was expressed as mean ± SD from four rats in each group at the end of the treatment. The data was statistically analyzed using ANOVAs with Turkey's post hoc test in the extracted and standard drug tests. Normal and pathological control were used to compare the significant difference in trypanosomal activity and the effect between the control and extract-treated groups. All statistical analyses were evaluated using SPSS version 2.0 software and Microsoft Excel. The value of $P < 0.01$ was considered a statistically significant difference (Emmanuel *et al*., 2011

RESULTS

The phytochemical composition of Garcinia kola aqueous seed extract

Table 1: Quantitative phytochemical component of *Garcinia kola* aqueous seed extract

Phytochemical component	Concentration (mg/100 g of *G. kola* dried seed powder)
Saponin	2.90 ± 0.05
Akanoids	13.30 ± 0.03
Tannins	4.05 ± 0.02
Anthraquinones	11.14 ± 0.01
Glycosides	4.05 ± 0.03
Flavonoides	2.05 ± 0.03
Carbohydrate	13.05 ± 0.02
Terpenes	3.05 ± 0.03
Steroids	36.13 ± 1.00
Quinines	0.06 ± 0.01

Data in triplicate mean ± S.D

The phytochemical screening of the aqueous extract of Garcinia kola revealed the presence of Saponin (2.90±0.05), Alkaloids (3.30±0.03), Taninins (4.05±0.02), Anthroquinones (1.14±0.01), Glycoside (4.05±0.03), Flavoids (2.05±0.03), Carbohydrate (13.05±00.02), Terpenes (3.05±0.03), Steroids (36.13±1.00) and Quinones (006±0.01). From the photochemical screening, Steroid has the highest concentration, 36.13±1.00/100 g, and Anthraquinones have the lowest, 1.4±0.01/100 g.

Table 2: the *In vitro* activities of *Garcinia kola* aqueous seed extract and Diaminizene aceturate observed on *T. brucei gambianse* parasite in 24 hours post incubation at 10^5/mL

Group	Group Treatment	Initial number of Parasite	3hours	6hours	9hours	12hours	15hours	18hours	21hours	24hours
A	200 mg of textract	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
B	400mg of extract	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
C	600mg of extract	5.00±0.00	5.00±0.00	4.00±0.00	4.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00
D	800mg of extract	5.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00	3.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
E	1000mg/of extract	5.00±0.00	3.00±0.00	2.50±0.00	1.00±0.00	0.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
F	1200mg/of extract	5.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
G	1400mg/ of extract	5.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
H (Standard drug control)	3.5mg/kg/bw of diaminizene aceturate	5.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Values in mean±SD

Garcinia kola seed extract possessed the highest *in vitro* activity in 1400 mg/kg/bw by reducing the trypanosome population from 5.0×10^5 to 0.0×10^5 in 6 hours of the post-incubation period. The standard drug also produced similar activity by reducing the parasite load from 5.0 105 to 0.00 105 at 3 hours. The least was produced in 200 mg, which reduced the parasite population from 5.0105 / ml to 4.0105/ml from 12 hours and remained static in 24 hours of the post-incubation period.

Table 3: Shows the *In vivo* activities of *Garcinia kola* aqueous seed extract and Diaminizene aceturate observed on *T. brucei gambianse* parasite at 10^5/mL in Trypanomiasis induced albino rat for 21 days treatment

Group	Group Treatment	Initial level parasite at 10^5/mL	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
A Normal control	10 ml of distilled water	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
B	200mg/kg/bw of extract	5.00±0.00	5.00±0.00	7.00±0.00	6.00±0.00	4.00±0.00	3.00±0.00	2.00±0.00	2.00±0.00
C	400mg/kg/bw of extract	5.00±0.00	5.00±0.00	7.00±0.00	5.00±0.00	4.00±0.00	3.00±0.00	2.00±0.00	1.00±0.00
D	600mg/kg/bw of extract	5.00±0.00	5.00±0.00	7.00±0.00	6.00±0.00	4.00±0.00	3.00±0.00	2.00±0.00	0.00±0.00
E	800mg/kg/bw of extract	5.00±0.00	5.00±0.00	4.00±0.00	3.00±0.00	3.00±0.00	1.00±0.00	1.00±0.00	0.00±0.00
F	1000mg/kg/bw of extract	5.00±0.00	5.00±0.00	3.00±0.00	2.50±0.00	2.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00
G	1200mg/kg/bw of extract	5.00±0.00	4.00±0.00	3.00±0.00	2.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
H	1400mg/kg/bw of extract	5.00±0.00	4.00±0.00	2.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
I Standard control	3.5mg/kg/bw of Diaminizine accturate	5.00±0.00	2.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
J Pathological control	10ml of Dextrose saline solution	5.00±0.00	6.00±0.00	8.00±0.00	10.00±0.00	20.00±0.00	Death	Death	Death

Values in mean±SD

When the *Garcinia kola* aqueous seed extract was tested for *in vivo* activities .3 the result revealed the reduction in parasitemia load in all the extract concentration test groups, 200, 400, 600, 800, 1000, 1200 and 1400 mg/kg/bw such that 200 mg/kg/bw of the extract reduced the parasitemia from $5.0\times10^5 \pm 0.00$ to $2.0\times10^5 \pm 0.00$ mean ± SD in 21 days which was the lowest concentration that produced trypanostatic effect on the parasite, 1400 mg/kg/bw of the extract shows to be highly effective by reducing the parasite load from $5.0 \times 10^5 \pm 0.00$ to $0.0 \times 10^5 \pm 0.00$ in 12 days post infection treatment in rat which produced a similar trypanocidal effect with 3.5 mg/kg/bw of Diaminizene aceturate at $5.0 \times 10^5 \pm 0.00$ to $0.0 \times 10^5 \pm 0.00$ in 3 days post infection treatment in rat when compared with the pathological control in which the parasitemia increased significantly from $5.0 \times 10^5 \pm 0.00$ to $20.0 \times 10^5 \pm 0.00$ in 12 days post infection treatment until the death of the rat were recorded due to parasitic effect from day 15 – 21 at ($p<0.01$).

DISCUSSION OF RESULTS

The *Garcinia kola* seed extract has been widely used in traditional medicine in Africa and Asia for various medicinal purposes; this present study evaluates the phytochemical composition, *in-vitro* and *in-vivo* anti-trypanosomal activates of *Garcinia kola* (Bitter kola) aqueous seed extract as an alternative replacement of Diamininzene aceturate drug in the treatment of Trypanosomiasis Disease using animal modeling. The phytochemical screening of the aqueous seed extract of *G. kola* revealed the presence of Saponin, Alkaloids, Tannins, Anthroquinones, Glucocide, Flavoids, Carbohydrate, Terpenes, Steroids, and Quinones. (Table 1) from the photochemical screening, Steroid has the highest concentration 36.13±1.00/100 g and Anthraquinones, has the lowest 1.4±0.01/100 g. This result is similar to the previous study of Auta *et al.* (2018), who reported that Garcinia kola seed contained alkaloid, 2.3±0.05, saponins, 2.42±0.04, flavonoids, 2.05±0.03, steroid, 31.13±1.00 respectively in 100g of powdered *Garcinia kola* seed extract. Findings from this study indicated that those phytochemicals may have contributed to the trypanocidal activity and its effect observed in the study compared with the standard drug. Different concentrations of the aqueous seed extract of *Garcinia kola* were studied for their *in-vitro* and *in-vivo* anti-trypanosomal activities against the *T. brucei gambianse* parasite and compared with Diamininzene aceturate drug. Compared with the standard drug, a decrease in the population of motile trypanosomes was taken as a measure of the *in-vitro* trypanosmal activities of the extract.

In Table 2, *Garcinia kola* seed extract had the highest *in-vitro* activity in 1400 mg/kg/bw by reducing the trypanosome population from 5.0×10^5 to 0.0×10^5 in 6 hours of post-incubation period. The least was produced in 200 g, which reduced the periodic population from 5.0105 /ml to 4.0105/ml in 24 hours of the post-incubation period. This observation compared well with Diaminizene aceturate, the standard trypanocidal drug employed as a control during the study, whose mechanism of action has been well established (Barett *et al*., 2002; Delepaux *et al*., 2007). The *Garcinia kola* seed extract has lower activity (*In-vitro*) at 3 to 24 hours in a lower concentration of 200, 400, and 600 mg. This may be due to the low concentration of active phytochemical components of the plant seed in these concentrations. However, its activities increase from 1000 to 1400 mg from 3 to 9 hours post-incubation. This result aligns with the previous study of Bulus *et al.* (2013), who reported that the extract of *Moringa olifera* stem and left had little effect on the parasite during a two-hour incubation. This variation could be partly attributed to differences in the type and amount of the photochemical in the various parts of the plant.

When the *Garcinia kola* aqueous seed extract was tested for *in-vivo* activities, Table 3. The result revealed a reduction in parasitemia load in all the extract concentration test groups, 200, 400, 600, 800, 1000, 1200, and 1400 mg/kg/bw, such that 200 mg/kg/bw of the extract reduced the parasitemia from $5.0\times10^5 \pm 0.00$ to $2.0\times10^5 \pm 0.00$ mean ± SD in 21 days which was the lowest concentration that produced a trypanocidal effect on the parasite, 1400 mg/kg/bw of the extract shows to be highly effective by reducing the parasite load from $5.0 \times 10^5 \pm 0.00$ to $0.0 \times 10^5 \pm 0.00$ in 12 days post-infection treatment in the rat which produced a similar trypanocidal effect with 3.5 mg/kg/bw of Diaminizene aceturate at $5.0 \times 10^5 \pm 0.00$ to $0.0 \times 10^5 \pm 0.00$ in 3 days post-infection treatment in rats when compared with the pathological control in which the parasitemia increased significantly from $5.0 \times 10^5 \pm 0.00$ to $20.0 \times 10^5 \pm 0.00$ in 12 days post-infection treatment until the death of the rats were recorded due to parasitic effect from day 15-21 at ($p<0.01$). This remarkable trypanocidal effect resembles the previous report of the study of Bulus et al. (2015), who reported that *Terminalia avicenniodis* plant extract resulted in significant suppression and total clearance of the parasitemia as observed by 14 days (P.I). This is as good as eliminating the parasite from animal blood, as seen with the Diaminizene aceturate treated group.

CONCLUSION

Trypanosomiasis is a disease of humans and livestock that affects the livelihood of Sub-Saharan Africans in rural and urban settlements. The research for a potent drug could help to reduce the scourge of African

trypanosomiasis as a result of adequate nonavailability of novel trypanocidal drugs, high toxicity, and non-cost effectiveness. Natural products are fast becoming an alternative method for the treatment of the disease because they are safer and cheaper; this current study revealed that *Garcinia kola* aqueous seed extract contains components that can effectively act on the *Trypanosoma brucie gambianse* parasite as a trypanostatics at lower concentrations < 400mg and trypanocidal at concentration > 400mg both in *in-vitro* and *in-vivo.*

RECOMMENDATION

These findings undoubtedly encourage the production of the present and future African chemotherapy that promises succor to a region that has suffered from the debilitating effect of trypanosomiasis and consequently improves the quality of life. There is a need for further collaborative study in this area, which intends to focus on the isolation, spectroscopic characterization, and pharmacokinetics of the bioactive ingredient in Garcinia kola aqueous seed, which may serve as a novel compound in the quest for the production of a new, affordable and more effective anti trypanocidal therapy.

Contribution to Knowledge

In contribution to knowledge, it was observed that Garcinia kola aqueous seed extract can be employed to design drugs for treating trypanosomiasis at lower concentrations. This can be applied as an alternative to Diaminizene aceturate drug in humans and animals for the treatment of trypanosomiasis disease as it was noticed that the extract possesses trypanosomatid and trypanocidal activates at different concentrations compared with the standard drug in the study.

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Availability of data statement:

All relevant information utilized for this study was obtained from Google Scholar data information base and it was utilized in the discussion of results and materials, and methods of this research work.

Authorship contributions

1 Abodun Moses oluwaseun contributed 60% of this research work, by formulating and propel the research

2 Oneyeweife Leonard C contributed 20% of this research work by prove reading the manuscript and carry out the necessary correction

3 Ekesiobi Anthony obinna contributed 20% of this research work by conducting the data analysis, interpretation and discussion of this research work