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Research Article

IN VITRO STUDY OF ANTITRYPANOSOMAL ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *GARCINIA KOLA* AGAINST *TRYPANOSOMA BRUCEI BRUCEI*

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ABSTRACT

Objective: The study was aimed at evaluating *in vitro* antitrypanosomal activity of *Garcinia kola* against *Trypanosoma brucei brucei*.

Methods: The plant materials were extracted and screened for phytochemicals. The antitrypanosomal activity of 70% and 100% *G. kola* extracts against the parasite was determined *in vitro*.

Results: Both extracts contained saponins, tannins, carbohydrates, cardiac glycosides, and flavonoids. However, anthraquinones and alkaloids were undetected. The parasites were seen to be actively motile within the first 30 min post-incubation period in 70% hydroethanolic *G. kola* extract and were found dead at 40–60 min in a higher concentration of extract. After 10 min post-incubation of the parasites in 100% ethanolic extract of *G. kola*, the parasites were observed to be active at lower concentration but at higher concentrations of the extracts, no trypanosomes were seen.

Conclusion: 100% extract induces ceasing of motility at the lesser time compared to 70% ethanolic extract. Similarly, the effect increases with increase in extract concentration. Further research should be carried out to elucidate the bioactive compounds present to have a broad knowledge on the mode of action of the compounds.

Key words: *Garcinia kola*, Phytochemicals, Trypanosomiasis, *Trypanosoma brucei brucei.*

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INTRODUCTION

Trypanosoma brucei brucei is a protozoan that causes trypanosomiasis in cattle and other domestic animal by infecting their blood plasma. This parasite is transmitted by tsetse flies of the family Glossinidae. The disease is prevalent in northern Nigeria. Susceptible animals when infected become weak, emaciate, and reproductively breeding animals may abort and become infertile [1]. Trypanosomiasis is a disease caused by several species of blood and tissue-dwelling protozoan parasites called trypanosomes which have a complex life cycle alternating between insect vector and the mammalian host [2]. Transmission of trypanosomes by insects may be affected by widely different means. Cyclical transmission, during which the trypanosomes actively multiply in thee vectors, occurs through an intermediary of *Glossina* or tsetse flies. Since the dawn of time, medicinal plants have been used traditionally to treat various kinds of diseases, [3-5] and today about 80% of the world's population relies mainly on traditional medicine for their primary health-care needs [6]. Hence, it seems rather justified that the research for new and improved plant-derived drugs for the treatment of trypanosomiasis should be further intensified. *Garcinia kola* is a dicotyledonous flowering plant in the Clusiaceae family. The genus contains only one species *G. kola*, which is found in West Africa particularly in Nigeria. Its trees are grown in the rainforests. *G. kola* has been identified as a potent antibiotic which could be effective in the treatment of many diseases. The development of resistance by the parasites over time due to the recurrent administration of drugs without proportional cure has posed the need to develop new drugs with a broader spectrum of action. The result obtained from the research will provide a safer, affordable, and effective substituent of trypanocidal drugs.

METHODS

Study area

The study was carried out at the Nigerian Institute for trypanosomiasis Research, Department of Trypanosomiasis and Vector and Parasitology Department, Ahmadu Bello University, Zaria, Kaduna State.

Collection of plant materials

A fresh leaf of *G. kola* was collected from Galma town, Giwa Local Government, Kaduna states Nigeria and was identified at the Herbarium section of the Nigeria Institute for Trypanosomiasis Research, Kaduna State, Nigeria.

Extraction of plant materials

The dried leaves were pounded to fine powder with a mortar and pestle. 100 g of powdered leaf of *G. kola* was extracted with 500 mL of absolute ethanol (100%). The mixture was vigorously shaken for 6 h and then allowed to stand for another 18 h. It was then shaken again and filtered using Whatmann filter paper (size 1). The filtrate was placed in an electric drier to evaporate slowly at 45°C to dryness [7].

Phytochemical screening of ethanol leaves extract of *G. kola*

The phytochemicals screened are as follows:

- Saponins: Frothing test was conducted according to the method of [8].
- Tannins: Lead sub-acetate test was conducted according to the method of [8].
- Alkaloids: Meyers test was conducted according to the method of [8].
- Cardiac glycosides: Keller-Killiani test was conducted according to the method of [8].
- Anthraquinones: Borntragers's test was conducted according to the method of [8].
- Flavanoids: Shinoda test was conducted according to the method of [8].
- Carbohydrates: Molisch's test was conducted according to the method of [8].

Test organism

T. b. brucei (Federer stain) was collected from the Department of Trypanosomiasis, Vector and Parasitology, National Institute for Trypanosomiasis Research, Kaduna State, Nigeria. Parasites were harvested from the blood of a donor rat which is at peak parasitemia.

Determination of parasitaemia

Parasitemia was monitored in blood collected from the tail. The number of parasites was determined microscopically by (×400) magnification using the rapid matching method of [9].

***In vitro* activity of crude extracts**

Assessment of *in vitro* antitrypanosomal activity of ethanol leaf extract of *G. kola* was performed in microtiter plates. A stock solution of 200 mg/mL of the ethanol leaf of *G. kola* was prepared. 200 µL of blood containing parasites (25 tryp/field) was incubated with equal volume of 200 µL of the ethanol leaf extract of *G. kola* solution of 20 mg/mL, 10 mg/mL, 5 mg/mL, and 1 mg/mL, respectively, to produce effective test concentrations of 10 mg/mL, 5 mg/mL, 2.5 mg/mL, and 0.5 mg/mL. A control was included which contained parasites suspended in 10 mg/mL of samorin in phosphate buffer saline only. The mixture was incubated at 37°C for 5 min in wells of microscope (×400) for a drop in cessation of motility at 10, 20, 30, 40, 50, and 60 min, respectively [10].

RESULTS AND DISCUSSION

Table 1 shows 70% hydroethanolic and 100% ethanolic extracts of *G. kola* contained saponins, tannins, carbohydrates, cardiac glycosides, and flavonoids. However, anthraquinones and alkaloids were not detected. The therapeutic and toxic effects of plant extracts on biological systems have been attributed to the actions of phytochemicals on these systems [11]. Some of these classes of compounds detected in the plant extracts used in this work have been shown to exhibit antitrypanosomal activities. With the exception of saponins which have been shown not to have antitrypanosomal actions [12], flavonoids are known to possess significant antitrypanosomal actions [13,14]. Therefore, the action of the extracts in eliminating or reducing the parasite motility may be due to the actions of flavonoids which are present in the extracts used in this study.

Among all four concentrations of extract used, the parasites were seen to be actively motile within the first 30 min post-incubation period in 70% hydroethanolic *G. kola* extract. The parasites were either not seen or were found lying dead after 40 min and 60 min at 20 mg/mL and 10 mg/mL concentrations, respectively. Reduced parasite motility was observed at the 50 min of 5 mg/mL and 1 mg/mL concentrations. The parasites were essentially dead at the 60 min of both concentrations of the extract used for incubating the parasites (Table 2).

At 10 min post-incubation of the parasites in 100% ethanolic extract of *G. kola*, the parasites were observed to be active at 1 mg/mL. However, at higher concentrations of the extracts, no trypanosomes were seen. After 30 min incubation period, the parasites were found lying dead (Table 3). The antitrypanosomal activities of *G. kola* have been evaluated by various research workers, and it has been demonstrated to confer significant antitrypanosomal activities [15]. Demonstrated the trypanostatic effect of 50% methanolic extract of *G. kola* in *T. b. brucei* infected rats at a dose of 600 mg/mL [13] showed that alkaloid fractions of *G. kola* were trypanocidal at 100 mg/mL. Some four depsipeptides from *Bionectria ochroleuca* obtained from *Sonneratia caseolaris* exhibited antitryponosomal activity against *T. brucei* [16].

CONCLUSION

The 70% and 100% ethanolic extracts of *G. kola* are confined with saponins, tannins, carbohydrates, cardiac glycosides, and flavonoids. Both extracts exhibit antitrypanosomal activity with 70% inhibiting motility after 50 min and after 20 min in 100% ethanolic extract. Similarly, the effect increases with increase in extract concentration. Further, research should be carried out to elucidate the bioactive compounds present to have a broad knowledge on the mode of action of the compounds.

Table 1: Phytochemical constituents of hydroethanolic and ethanolic extracts of G. kola

Classes of phytochemicals	70% G. kola	100% G. kola
Saponins	+	+
Alkaloids	-	-
Tannins	+	+
Anthraquinones	-	-
Flavonoids	+	+
Carbohydrates	+	+
Glycosides	+	+

+: Present -: Absent. *G. kola: Garcinia kola*

Table 2: Observed T. b. brucei motility after incubation in 70% hydroethanolic extract of G. kola

Concentration (mg/mL)	10 min	20 min	30 min	40 min	50 min	60 min
1	++++	++++	++++	+++	++	**
5	++++	++++	+++	+++	++	**
10	++++	+++	+++	*	**	**
20	+++	+++	+++	*	*	**
Control	*	*	*	*	*	*

*No parasite but red blood cells, **Parasites lying dead, +weak parasites, ++slightly weak parasites, +++actively motile parasites, ++++very active motile parasites, *G. kola: Garcinia kola,* T. brucei brucei: Trypanasoma brucei brucei

Table 3: Observed T. b. brucei motility after incubation in 100% ethanolic extract of G. kola

Concentration (mg/mL)	10 min	20 min	30 min	40 min	50 min	60 min
1	+++	**	**	*	*	*
5	**	**	**	*	*	*
10	**	**	**	*	*	*
20	**	**	**	*	*	*
Control	*	*	*	*	*	*

*No parasite but red blood cells, **parasites lying dead, +weak parasites, ++slightly weak parasites, +++actively motile parasites, ++++very active motile parasites

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