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In vitro Anti-trypanosomal activity of curcumin isolated from *Curcuma longa* (Turmeric) rhizomes

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Abstract
The exponential rate of cell divisions observed in African trypanosomes has prompted the hypothesis that substances with potent anti-proliferative potential against cancer cells may exhibit anti-trypanosomal activities. Curcumins have been reported to inhibit cancerous cell proliferation in animal models. In the present study, the *invitro* anti-trypanosomal potential of curcumin isolated from rhizomes of *Curcuma longa* was evaluated against *T. b. brucei*, Federe strain. The method of was adopted in the extraction of curcumin from powdered Rhizomes of *Curcuma longa*. The *invitro* assay, conducted in 96-well titre plate, over test concentrations of 0.156 and 10 mg/ml, revealed that Curcumin demonstrated anti-trypanosomal activity after 8 hours of incubation, with significant reduction of trypanosome counts in wells containing various concentrations of the isolate compared to the control wells.

Keywords: *Curcuma longa, Curcumins, antitrypanosomal activity, T. b. brucei, Turmeric, Trypanosomiasis*

Introduction
Human African Trypanosomiasis (sleeping sickness) is a debilitating vector-borne parasitic disease. Listed by the World Health Organization as one of the neglected tropical diseases (NTDs), it is a major constraint to human and livestock development consequently leading to food insecurity especially in Sub Saharan Africa. Trypanosomiasis is caused by trypanosomes, which are protozoans of the Phylum *Sarcomastigophora*, Order *Kinetoplastida*, Family *Trypanosomatidae* and Genus *Trypanosoma.* [2] Among the numerous species of *Trypanosoma*, *T. brucei* of great economic importance. *T. brucei gambiense* is responsible for chronic form of Human African Trypanosomiasis in West and Central Africa. *T. brucei rhodesiense* is the etiological agent responsible for acute form of the disease in East Africa, while *T. brucei brucei*, which causes virulent infection in mice, also serves as a model for laboratory studies. Left untreated, the disease is responsible for substantial morbidities and mortalities [3].
Available drugs are antiquated, toxic, and compromised by emerging resistance [4]. Thus the need for non toxic, natural agents as alternatives. This study seeks to investigate the antitrypanocidal activities of Curcumin which is a bright yellow-colored Indian spice. This spice has been used for centuries to treat numerous diseases such as diabetes, atherosclerosis as well as liver, rheumatoid, and infectious diseases [5]. Curcumin, is also known for various biological activities, including its broad-spectrum antibacterial nature and its membrane damaging property probably due to its antioxidant mechanism [6]. Curcumin, is a highly bioactive and important component of turmeric.7 Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family which has had great success as a therapeutic agent against many ailments and infections, from cancer to inflammations [7, 8] *Curcuma longa* has been traditionally used in Asian countries as a medical herb due to its antioxidant, anti-inflammatory, antimutagenic, antimicrobial and anticancer properties [8]
However, a direct assay of pure curcumin has scarcely been carried out for its anti Trypanosoma activities. Hence, the object of this study is to evaluate the antitrypanosomal activity of curcumin against *Trypanosoma brucei brucei, in vitro*.

Materials and Methods
Plant Material: The powdered turmeric used was procured from Yakubu Farms, Ungwan Rimi, Kaduna, Nigeria.

Extraction of curcumin from the turmeric rhizome powder: The method of 1 was used in the extraction of curcumin from the turmeric rhizome, with slight modifications. 25g of pulverized turmeric rhizome was initially macerated in 1 liter of n-Hexane for 72 hours, with intermitted stirring. The suspension was filtered through a glass funnel fitted with No. 1 Whatman filter paper. The marc was air dried and further subjected to soxhlet extraction in 310ml of methanol. The distillate was evaporated to dryness via open air evaporation. The crude extract obtained was dissolved in 100 mL of 0.2 M NaOH, followed by the addition of 100 mL of toluene; the mixture was poured into a separation funnel and inverted several times. The brown colored aqueous phase containing NaOH was collected and acidified to pH 3 by the addition of 0.2 M HCl to clarify the aqueous phase, yielding a yellow coloured solution/emulsion. The clarified solution was then extracted with diethyl ether (3 x 100mL) in a separating funnel. The combined ethereal phases were washed with 30 mL of distilled water in a separating funnel and air dried. The ether fraction (0.65g) containing curcumin was stored at 4 °C until required.

Test organism: Stabilate of *Trypanosoma brucei brucei* (Federe strain) was obtained from the Cryobank maintained at the Vector and Parasitology Studies Department of the Nigerian Institute for Trypanosomiasis Research, Kaduna, Nigeria. The stabilate was thawed at 37ºC and trypanosomes were screened for viability by examining wet smears prepared from the stabilates in the light microscope at x400 magnification. The presence of motile trypanosomes was taken as indication of trypanosome viability.

Donor animals: Population of viable trypanosomes was expanded in mice. Blood suspension containing the viable trypanosomes was inoculated into two mice (donor animals) via the intraperitoneal route. The inoculated animals were screened for infection with trypanosomes by examining wet smears of blood prepared from a drop of blood collected from the tail of the inoculated mice. At raising parasitemia, the mice were euthanized following chloroform anesthesia and the blood collected via cardiac puncture into EDTA tubes.

Reconstitution of isolated curcumin and reference drug (Diminazene Aceturate): RPMI 1640 medium used for the *in vitro* assay was supplemented with heat inactivated goat serum 10% (v/v), 1% (w/v) glucose, and gentamycin 40µg/ml [9]. Solutions of the curcumin isolate and drug (Diminazene aceturate) were reconstituted in supplemented medium as follows: stock solution of the isolate was prepared by dissolving 10 mg of the isolate in 1 ml of supplemented medium. Subsequently, the stock solution was serially diluted in supplemented medium to yield various concentrations of the isolate ranging from 1 mg/ml to 0.03125 mg/ml. Similarly, various concentrations of Diminazene aceturate were reconstituted, ranging from 1 mg/ml to 0.03125 mg/ml [3]

***In vitro* anti-trypanosomal assay:** 100µl of the reconstituted solutions of the curcumin isolate, as well as the reference drug (Diminazene aceturate), were separately dispensed in duplicate into wells of a 96-well microtitre plate. 30µl of the blood suspension containing *T. b. brucei* was added to each of these wells and gently mixed together. Control wells containing only 100µl complete medium and 30µl blood suspension were also included. The micro titre plate was placed in a candle jar containing 5% CO_2 within an incubator. Wet smears were prepared from each of these wells 8 hours post-incubation; each smear was examined in the light microscope (X400 magnification) for alterations in trypanosome motility and counts over three fields of view per smear, a total of six observations per concentration [3].

Statistical analysis: Data was analyzed using Statistical Package for Social Science (SPSS) version 20 software. Descriptive data are given as mean ± standard error of mean (SEM) of trypanosome counts and line graphs. The mean trypanosome count per concentration of extracts at the various time intervals of incubation were compared using one-way analysis of variance (ANOVA) and student paired *t* test at significance level of $p< 0.05$.

Results

Antitrypanosomal effect of curcumin: Shown in Table 1 are the mean counts of trypanosomes 8 hours post incubation in graded concentrations of curcumin and Diminazene aceturate. The mean trypanosome counts in wells containing curcumin were significantly different from that of the control over the entire range of the test concentrations, 8 hours post-incubation. Between 10 and 0.3125 mg/ml concentrations of curcumins, 100 percent mortality was recorded with no surviving trypanosomes seen in wet smears. At 0.15625 mg/ml concentration, the mean count was 5.50 ± 0.50 per field (56.6% mortality) while in the negative control wells it was 12.5 ± 0.99.

Antitrypanosomal effect of Diminazene aceturate: Exposure of trypanosomes to 10 and 5.0 mg/ml concentrations of Diminazene aceturate resulted in 100 percent mortality. Between 2.5 and 0.15625 mg/ml Diminazene, significant ($p< 0.05$) concentration dependent decline in number of surviving trypanosomes was recorded (Table 1).

Comparative sensitivity of *Trypanosoma brucei brucei* to the effects of isolated *Curcumin* and Diminazene aceturate: There was no statistically significant difference ($p > 0.05$) in the mean trypanosome count in wells contain Diminazene or *Curcumin* at 10 and 5 mg/ml concentrations. Statistically significant differences ($p< 0.05$) were, however, observed in their effects between 1.25 and 0.3125 mg/ml concentration (Table 1).

Table 1: The effect of isolated *Curcumin* and Diminazene Aceturate on trypanosome count

S/No	Concentration (mg/ml)	*Curcumin*	Diminazene	*p* value (t test)
1	10	0.00 ± 0.00^a	0.00 ± 0.00^a	-
2	5.0	0.00 ± 0.00^a	0.00 ± 0.00^a	-
3	2.5	0.00 ± 0.00^a	0.17 ± 0.17^a	0.363
4	1.25	0.00 ± 0.00^a	1.83 ± 0.60^b	0.010
5	0.625	0.00 ± 0.00^a	2.67 ± 0.67^b	0.028
6	0.3125	0.00 ± 0.00^a	2.67 ± 0.49^b	0.003
7	0.15625	5.50 ± 0.50^b	4.00 ± 0.82^b	0.251
8	Control	12.5 ± 0.99^c	12.5 ± 0.99^c	-

The values are expressed as mean ± standard error of mean (SEM). In each row, mean values with different superscripts are statistically significant ($p< 0.05$).

Discussion

Premised upon its anti-proliferative activity against cancer cells lines, curcumin, extracted from rhizomes of *Curcuma longa* (Turmeric), was evaluated for it's *in vitro* antitrypanosomal activity against *Trypanosoma brucei brucei* (Federe strain). The results, shown in Table 1, indicate that curcumin demonstrated significant ($p< 0.05$) antitrypanosomal activity *invitro*. Our findings agree with those of [10] who reported that extracts of curcuma longa rhizome exhibited moderate antitrypanosomal activity.

Between 10 and 0.156 mg/ml concentrations of the curcumin isolate, trypanosomes with intact morphologies were not seen in wet smears prepared from titre plates. This may imply that the observed effect of curcumin on trypanosomes may be mediated by membrane acting agents which, solubilizing the membrane of trypanosomes resulted in subsequent cellular lysis. The activity of curcumin could also have been mediated through intracellular mechanisms which may impair cell membrane integrity or alter osmotic balance to favour cell rupture.

The trypanocidal drug, Diminazene aceturate, also demonstrated significant antitrypanosomal activity against *T. b. brucei*, *in vitro*. The effect of the trypanocide was concentration dependent with increasing activity associated with increasing concentrations of the drug. When compared with the test substance (curcumin), it was observed that Diminazene exhibited significantly lower antitrypanosomal activity over 10 and 0.3265 mg/ml concentration. This difference in observed antitrypanosomal activity may be due to the differences in mechanism of action (MOA) of the two agents. While the MOA of curcumin against trypanosomes has not been elucidated, Diminazene is reported to bind to Kinetoplast DNA *in vitro* and interfere with nucleic acid biosynthesis [11, 12]. Such binding events would require a much longer duration for its effect to be observed than the period of incubation adopted (8 hours) for this study [13]. Have reported a mean doubling time of 6.8 hour for *Trypanosoma brucei brucei* BS427. This may be responsible for the differential in antitrypanosomal activities due to Diminazene Aceturate and the isolated curcumin.

Conclusion

Curcumin from rhizomes of *C. longa* contains active principles with significant *in vitro* antitrypanosomal activity against *T. b. brucei* (Federer strain) between 10 and 0.156 mg/ml concentration.

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