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Research Article

Assessment of *InVitro* Antitrypanosomal Effects of *Terminalia Catappa* Leaf Extract and Fractions on *Trypanosoma Brucei Brucei*

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Abstract

Due to the high cost of conventional drugs used in the treatment of trypanosomosis, the drive towards ethnomedicine has become necessary. The aim of this study was to assess the *in vitro* antitrypanosomal effects of *Terminalia catappa* leaf extract and fractions on *Trypanosoma brucei brucei*. The extract and fractions of *T. catappa* leaves were prepared using standard laboratory method. Culture of *T. brucei brucei* was done using 96-well microtiter plate in triplicates and maintained at 37^0C. About 20-25 parasites were dosed with 0.4, 2 and 4mg/ml of leaf extract and fractions (n-hexane and ethylacetate) and a control without extract followed by incubation in Eppendorf tubes. Results revealed parasites survival over 4 hours in control well. There was complete cessation of parasite motility within 60 minutes by diminazene aceturate and ethylacetate fraction (EAF) at 4 and 2 mg/ml. The crude extract and n-hexane fractions (HF) produced significant decrease in parasites motility after 15 and 10 minutes respectively at 4 mg/ml; after 20 and 15 minutes respectively at 2 mg/ml. At 0.4 mg/ml, parasites motility was significantly decreased by only EAF within 60 minutes. The leaves of *T. catappa* possess *in vitro*

antitrypanosomal activity with the EAF being most effective at lowest concentration.

Keywords: *In vitro*; Antitrypanosomal; *Terminalia catappa;* Extract; Fraction; Motility

1. Introduction

Globally, the use of plants and plant-derived compounds in prophylaxis and therapy against diseases has been documented (Triantafillidis et al. [1]). *Terminalia catappa* being a large tree belongs to the family *combretaceae* and is native to tropical regions of Asia, Africa and Australia (Ramachandra et al. [2]; Anand et al. [3]). This tree is grown for its ornamental purposes and its edible nuts whose kernel can be eaten raw (Anand et al. [3]). The leaves and barks have been used in herbal medicines for various purposes such as medicinal lotion for leprosy and scabies, relieve of stomachache and headache. In Taiwan, fallen leaves of *Terminalia catappa* have been used in the treatment of liver diseases (Ratinasoriya and Dharmasiri, 2000 [4]).

Several pharmaceutical studies of *Terminalia catappa* have demonstrated the presence of essential medicinal phytoconstituents possessing biological activities which support its traditional uses (Pandya et al. [5]; Yeh et al. [6]). These medicinal constituents include phenol, flavonoid, and carotenoid. The activities reported for the leaf extract include antioxidant and hepato-protective (Kinoshita et al. [7]), antiviral (Tan et al. [8]), anti inflammatory (Fan et al. [9]), antidiabetic (Anand et al.[3]), anticancer (Naitik et al. [10]), antimicrobial (Taganna et al.[11]), antifungal (Terças et al. [12]) and wound-healing (Khan et al. [13]).

Trypanosomosis, a protozoan disease, still continues to threaten the economic and social wellbeing of sub-Saharan Africans and there is need for effective treatment (Odeniran and Ademola [14]). Also, the conventional trypanocidal drugs do produce toxic effects in animals (Melaku and Birasa [15]) and safer alternative drugs discovery would improve livestock production. These alternatives including medicinal plants have been utilized traditionally at unregulated amounts. With the above mentioned biological activities of *Terminalia catappa*, its efficacy in the treatment of trypanosomosis will be beneficial. Hence, in this study, the *in vitro* antitrypanosomal effects of *Terminalia catappa* leaf extract and fractions on *Trypanosoma brucei brucei* were assessed.

2.Materials and Methods

2.1 Plant collection

Leaves of *Terminalia catappa* collected from the Botanical Garden, Ahmadu Bello University (ABU) Zaria, Nigeria, were identified by a taxonomist at the Department of Botany herbarium unit, ABU Zaria. Voucher specimen was deposited under the reference number 1556.

2.2 Extract Preparation and Fractionation

The collected fresh leaves of *Terminalia catappa* were air-dried for 3 weeks followed by grounding into fine powder using mortar and pestle. The ground material was macerated in ethanol for 4 days, microwaved and filtered. The filtrate was evaporated using rotary evaporator. The crude extract (CE) was partitioned into n-hexane (HF) and ethylacetate (EAF) fractions, and fractions were concentrated by evaporation using rotary evaporator. The crude extract

and fractions were then used for *in vitro* antitrypanocidal screening.

2.3 *Trypanosome* Parasite

Trypanosoma brucei brucei was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna. The parasite was maintained in the laboratory by continuous passage in mice until required. Passage was considered necessary when parasitaemia was in the range of 16-32 parasites per field. An aliquot of 0.2ml of the parasitized blood diluted with phosphate buffered saline (PBS) to contain approximately 1 $x10^3$ parasites/ml was injected into clean rats which was acclimatized under laboratory conditions for about two weeks.

Blood was obtained from the tail of rats and parasitaemia monitored by determining the number of parasites microscopically at × 400 magnification using the "Rapid Matching" method described by Herbert and Lumsden (1976).

2.4 *In vitro* antitrypanosomal activity

The test was carried out using the method as described by Atawodi et al. [16] with slight modification in triplicates. Ten grams of the plant extracts weighed into Eppendorf tubes was initially dissolved in 0.1ml of 20% dimenthyl sulfoxide (DMSO) and extract concentration of 10.0 mg/ml prepared for stock extract solution by appropriate dilution with phosphate buffer saline (PBS). Assessment of *in vitro* anti-trypanosomal activity was performed in 96-well microtitre plates. Two effective test concentrations of 4 mg/ml and 2 mg/ml in well of microtitre plates were prepared by mixing 20µl of blood containing about 20-25 parasites with 5 µl of extract solutions of 20.0mg/ml and 10.0mg/ml respectively. Also, sets of controls containing the parasites suspended in about 2% DMSO only and diaminazene aceturate were included. All these were incubated in closed Eppendorf tubes maintained at 37℃.

Following incubation for 5 minutes, 2µl of test mixtures were placed on separate microscope slides, covered with cover slips and parasites were observed every 5 minutes for a total duration of 60 mins. Drop in motility of parasites in blood treated with extract compared to blood without extract was taken as a measure of anti-trypanosomal activity (Atawodi et al. [16].

3. Results

At 4 mg/ml, parasites motility was completely ceased (EAF) and slowly decreased (HF) after 5 minutes; decreased significantly (HF) and slowly decreased (CE) after 10 minutes and decreased significantly by CE after 15 minutes (Table 1).

At 2 mg/ml, parasites motility slowly decreased (CE) after 15 minutes; decreased significantly after 10 minutes (EAF), 15 minutes(HF) and 20 minutes (CE); and completely ceased after 15 minutes by EAF (Table 1).

At 0.4 mg/ml, parasites motility was slowly decreased by HF after 55 minutes and decreased significantly by EAF after 30 minutes. No effect on the parasites was observed by the crude extract at this concentration within 60 minutes by CE. (Table 1).

Table 1: *In vitro* antitrypanosomal activity of crude extract and fractions of *Terminalia catappa* leaves against *Trypanosoma brucei brucei.*

Leaf extract and fractions	**Time (minutes) after which motility of parasites was affected**		
	4 mg/ml	2 mg/ml	0.4 mg/ml
Crude extract	10^{SD}	15^{SD}	-
	15^{DS}	20^{DS}	
n-Hexane fraction	5^{SD}	15^{DS}	-
	10^{DS}		55^{SD}
Ethylacetate fraction	5^{CC}	10^{DS}	-
		15^{CC}	30^{DS}
Diminazene aceturate	5^{CC}	5^{CC}	5^{DS}
			10^{CC}

Key:

- = no effect on motility after 60 minutes

SD = slowly decreased mortality

DS = decreased motility significantly

CC = ceased motility completely

4. Discussion

From this study, the crude extract and fractions of *Terminalia catappa* leaves demonstrated various antitrypanosomal activities with more activities observed by ethylacetate fraction even at the minimum concentration of 0.4 mg/ml. In a study by Bala et al. [17] using *T. catappa* stem bark extract, *T. b. brucei* motility was ceased after 55 minutes at 4 mg/ml, drastically reduced at 2 mg/ml and 1 mg/ml after 20 and 35 minutes respectively. The variation in time after which parasites motility was affected could be due to the part of the plant used.

This suggests that the leaf extract and fractions were more active in affecting motility in our study as parasite motility was ceased after 5 minutes and 15 minutes by EAF at 4 mg/ml and 2 mg/ml respectively. Abiodun et al. [8] reported antitrypanosomal activity of *T. catappa* and *T. orientalis* leaf extracts against *T. b. rhodiesiense* with lowest inhibitory concentration-50 (IC_{50}) recorded by the EAF (7.80 ± 1.83 µg/ml; 3.50 ± 0.59 µg/ml). This suggests that the EAF of *T. catappa* leaves was more potent in producing antitrypanosomal activity compared to other extracts, fractions and parts of the plant.

The antitrypanosomal activity of *T. catappa* in this study could result from the action of flavonoids, terpenes and hydrolyzable tannins which include ellagic acid, flavogallonic acid, punicalagin, and terchebulin (Shuaibu et al. [19]). The potential of these compounds in killing or inhibiting the growth of

various trypanosomes have been reported in numerous *in vitro* studies. A suggested mechanism of this antitrypanosomal activity could be due to inhibition of the trypanosome alternative oxidase (TAO) enzyme (Yabu et al. [20]; Saxena et al., 2013; Mergia et al. [21]). Metals chelation which in turn inhibit lipid peroxidation in trypanosomes by flavonoids and flavonoid-derived products have also been suggested to contribute to antitrypanosomal activity (Harbourne and Williams [22]; Mergia et al. [21].

Tannins have been suggested to form complexes with proteins through non-specific forces thus resulting in decreased parasite motility (Taylor [23]). The mechanisms of action by terpenes include aldehyde-thiol adducts formation leading to decreased buffering agents thus creating oxidative stress in cells (Nibret et al. [24]); oxidation of glutathione, pyruvic and alpha-ketoglutaric acids and the oxidative decarboxylation of pyruvic acid by hydroperoxy group which makes them toxic (Saeidnia et al. [25]).

From this study, leaves of *T. catappa* possess *in vitro* antitrypanosomal activity with the ethylacetate fraction being the most effective. Whether the antitrypanosomal activity is attributed to result from either the individual class of compounds, or the synergistic effect exerted by the compounds could not be speculated. Hence, further studies are required to establish the particular mechanism by which *T. catappa* produced *in vitro* antitrypanosomal activity.

Conflict of Interest

The authors declare no conflict of interest.

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