

IN VITRO ANTITRYPANOSOMAL ACTIVITY OF METHANOLIC LEAF EXTRACT OF SENNA OCCIDENTALIS AGAINST TRYPANOSOMA BRUCEI-BRUCEI

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ABSTRACT

African trypanosomiasis remains a major constraint to human and livestock health, and existing trypanocides are compromised by toxicity, cost, and increasing drug resistance, creating a need for alternative agents from medicinal plants. This study evaluated the phytochemical constituents and *in vitro* antitrypanosomal activity of the methanolic leaf extract of *Senna occidentalis* against *Trypanosoma brucei*. Fresh leaves were collected from Samaru, Zaria, dried, and extracted by maceration in absolute methanol. Phytochemical screening was conducted using standard methods, and antitrypanosomal activity was assessed using the Drug Incubation Survival Test against graded extract concentrations (0.16-10 mg/ml), with Diminazene aceturate as the reference drug. The extract yielded 7.0 g of a dark green, gummy residue and tested positive for flavonoids, tannins, alkaloids, saponins, and steroids/terpenes, but negative for cardiac glycosides. Trypanosome survival declined significantly with increasing extract concentration ($p < 0.05$), with complete loss of motility at 5.0 and 10 mg/ml six hours post-incubation; the median lethal concentration (LC_{50}) of the extract was 0.71 ± 0.04 mg/ml, lower than 0.10 ± 0.02 mg/ml for the reference drug. These findings indicate that the methanolic leaf extract of *S. occidentalis* possesses an appreciable antitrypanosomal activity attributable to its phytochemical constituents, supporting its potential as a natural source for trypanocidal drug development, pending further *in vivo* evaluation.

Keywords: *Senna occidentalis*; *Trypanosoma brucei-brucei*; phytochemical screening; antitrypanosomal activity; median lethal concentration; medicinal plants.

INTRODUCTION

Trypanosomiasis is a blood-borne protozoan parasitic disease affecting humans and animals. It is a major hindrance to livestock health and production across Africa (Rascón-García *et al.*, 2023). The disease is caused by several *Trypanosoma* species, including *Trypanosoma brucei*, which is associated with animal trypanosomiasis. Infection can result in disease, reduced animal productivity, and, in severe cases, death (Odeniran *et al.*, 2018). Treatment of animal trypanosomiasis relies on limited trypanocidal drugs, and the continued use of these drugs has raised concerns about drug resistance and reduced treatment effectiveness, creating a need to identify new compounds with trypanocidal activity (Ungogo & de Koning, 2024). The limited number of available treatments makes it necessary to explore alternative sources of trypanocidal compounds.

Medicinal Plants remain an important source of drugs, particularly

in traditional medicine, because they have several naturally occurring bioactive compounds. In many African communities, medicinal plants play an important role in healthcare, especially where access to conventional healthcare and medicines is limited (WHO, 2019). Medicinal plants have also been investigated as potential sources of new anti-trypanosomal agents (Nekoei *et al.*, 2022).

Senna occidentalis (Linn.) (formerly *Cassia occidentalis*) is a weed of the family Leguminosae distributed throughout the tropical and subtropical regions of the world and is among the most widely used herbal plants in these regions (Veronique and Gabriel, 2013), with its roots, leaves, flowers, and seeds used for various therapeutic purposes in traditional medicine. Alhafiz *et al.* (2020) found that methanolic extracts from different plant parts inhibited *Trypanosoma brucei*, with the root extract showing the highest activity.

Although these findings provide evidence for the anti-trypanosomal potential of *S. occidentalis*, the activity of a plant extract can vary depending on the plant part used, extraction procedure, extract concentration, parasite preparation, and assay conditions. This study therefore evaluated the phytochemical constituents and *in vitro* anti-trypanosomal efficacy of the methanolic leaf extract of *S. occidentalis* against *Trypanosoma brucei*, with the specific objectives of determining the extract's qualitative phytochemical constituents, assessing its anti-trypanosomal activity *in vitro*, and evaluating the effects of lethal concentrations on trypanosome motility.

MATERIALS AND METHODS

Sample Collection and Identification

Fresh leaf samples of *S. occidentalis* were obtained from Samaru, Zaria, Sabon Gari Local Government Area, Kaduna State. The plant was identified and authenticated at the Herbarium Unit, Department of Botany, Ahmadu Bello University, Zaria, and assigned Voucher number ABU060125; a specimen was deposited for reference.

Sample Preparation and Extraction

Harvested leaves were dried in a hot-air oven to prevent thermal degradation of active components and then ground to a powder using a laboratory mortar and pestle. Seventy-five grams (75 g) of the powdered material was macerated in 400 mL of absolute methanol (1:3) for 48 hours, filtered, and the filtrate concentrated over a water bath. The resulting extract was stored at room temperature until required.

Phytochemical Analysis

The methanolic leaf extract was screened for flavonoids, tannins, alkaloids, steroids, terpenes, saponins and cardiac glycosides using qualitative phytochemical screening methods with reference to Alkali *et al.* (2024) and Badock *et al.* (2024). Colour changes and precipitate formation were observed and recorded for each test.

Parasite Source and Donor Animals

A cryopreserved stablate of *T. b. brucei* was obtained from the Nigerian Institute for Trypanosomiasis Research, Kaduna, warmed to 37 °C, and screened for viability by wet-smear using an Olympus CX21 light microscope (Olympus Corporation, Tokyo, Japan) at ×400 magnification. Two albino Wistar rats were inoculated intraperitoneally with 0.2 ml of a blood suspension prepared from the stablate, and parasitaemia was monitored daily until it reached approximately 10⁹ parasites/ml, after which parasitised blood was collected by cardiac puncture into EDTA sample containers (Bulus and Addau, 2013). Parasitaemia was estimated using the rapid matching method described by Herbert and Lumsden (1976).

In Vitro Antitrypanosomal Assay

RPMI 1640 medium supplemented with gentamycin (40 µg/L), 10 % heat-inactivated goat serum, and 1 % glucose was used for the assay. Stock solutions (10 mg/ml) of the extract and the reference drug (Diminazene aceturate) were serially diluted to give concentrations from 10 to 0.3125 mg/ml. The Drug Incubation Survival Test was used: 100 µl of each extract or drug concentration was dispensed in duplicate into wells of a 96-well microtitre plate, and 20 µl of parasitised blood suspension was added to each well; control wells received medium and blood suspension only. Plates were incubated at 37 °C in approximately 5 % CO₂. Wet smears were prepared 20 hours post-incubation and motile trypanosomes counted by light microscopy (×400) across three fields per well (nine observations per concentration). The reduction in motile trypanosome counts relative to the control was taken as the index of antitrypanosomal activity.

Data Analysis

Data were analysed by analysis of variance (ANOVA) and are presented as mean ± standard error of the mean.

RESULTS

Physical Properties and Phytochemical Constituents

The methanolic leaf extract of *S. occidentalis* yielded 7.0 g of a dark green, gummy residue (Table 1). Phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, saponins, and steroids/terpenes, while cardiac glycosides were not detected (Table 2).

Table 1: Physical properties of *Senna occidentalis* methanolic leaf extract

Plant part	Type of extract	Yield weight, texture, colour
Leaf	Methanolic extract	7.0 g; gummy; dark green

Table 2: Phytochemical constituents of *Senna occidentalis* methanolic leaf extract

Sample	Parameter	Inference
<i>Senna occidentalis</i> (methanolic extract)	Flavonoids	+
	Tannins	+
	Alkaloids	+

Saponins	+
Cardiac glycosides	-
Steroids and terpenes	+

+ = Presence; - = Absence

Effect of Graded Concentrations of the Extract on Mean Trypanosome Count

The mean number of motile trypanosomes after six hours of incubation declined progressively with increasing concentration of both the extract and the reference drug, and the decrease was statistically significant ($p < 0.05$; Table 3). In the negative control, the mean count of surviving trypanosomes was 24.3 ± 0.67 per field, falling to 1.00 ± 0.52 per field at 2.5 mg/ml of extract, with no motile trypanosomes detected at 5.0 and 10 mg/ml; the minimum trypanocidal concentration of the extract was therefore 5.0 mg/ml at six hours post-incubation. For Diminazene aceturate, no motile trypanosomes were detected at concentrations of 0.625 mg/ml or higher, indicating a minimum trypanocidal concentration of 0.625 mg/ml.

Table 3: Mean number of surviving trypanosomes after six hours of incubation

Concentration (mg/ml)	<i>Senna occidentalis</i>	Diminazene aceturate
0.0 (Control)	24.3 ± 0.67 d	24.8 ± 1.28 b
0.15625	18.8 ± 0.54 c	7.33 ± 0.61 b
0.3125	17.0 ± 0.45 c	6.50 ± 0.43 b
0.625	16.3 ± 0.61 c	0.00 ± 0.00 a
1.25	10.8 ± 0.91 b	0.00 ± 0.00 a
2.5	1.00 ± 0.52 a	0.00 ± 0.00 a
5.0	0.00 ± 0.00 a	0.00 ± 0.00 a
10	0.00 ± 0.00 a	0.00 ± 0.00 a
p value	< 0.0001	< 0.0001

Values are mean ± standard error of the mean. Within each column, means sharing a letter do not differ significantly ($p < 0.05$).

Percentage Mortality and Median Lethal Concentration (LC₅₀)

The percentage mortality of trypanosomes increased with the concentrations of both the extract and the drug, paralleling the trend in surviving trypanosome counts (Table 4). A t-test showed significant differences ($p < 0.05$) between extract- and drug-associated mortality at concentrations from 0.15625 to 2.5 mg/ml. In contrast, at 5.0 and 10 mg/ml, the extract and drug produced comparable effects ($p > 0.05$). The LC₅₀ of the extract after six hours of incubation was 0.71 ± 0.04 mg/ml, compared with 0.10 ± 0.02 mg/ml for the reference drug, a statistically significant difference ($p < 0.05$).

Table 4: Percentage mortality of trypanosomes and median lethal concentration (LC₅₀) after six hours of incubation

Concentration (mg/ml)	<i>Senna occidentalis</i>	Diminazene aceturate
0.0 (Control)	-	-
0.15625	18.4 ± 6.71 a	68.3 ± 6.23 b
0.3125	26.2 ± 8.70 a	71.6 ± 6.16 b
0.625	30.5 ± 3.36 a	100 ± 0.00 b
1.25	51.4 ± 10.4 a	100 ± 0.00 b
2.5	95.8 ± 5.18 a	100 ± 0.00 b
5.0	100 ± 0.00 a	100 ± 0.00 a
10	100 ± 0.00 a	100 ± 0.00 a
LC ₅₀ (mg/ml)	0.71 ± 0.04 b	0.10 ± 0.02 a

Values are mean $\pm$ standard error of the mean. Within each row, means sharing a letter do not differ significantly ($p < 0.05$).

DISCUSSION

The phytochemical screening of the methanolic leaf extract of *S. occidentalis* revealed the presence of flavonoids, tannins, alkaloids, saponins and steroids/terpenes, while cardiac glycosides were not detected. These findings are consistent with other reports on *S. occidentalis* (Alhafiz *et al.*, 2020; Mohammed & Alhassan, 2017). However, Isah *et al.* (2018) reported the absence of alkaloids and the presence of cardiac glycosides in an aqueous leaf extract of *S. occidentalis*. Kabila *et al.* (2017) also reported differences in the phytochemical composition of *S. occidentalis* leaf extracts. These differences may be related to the extraction solvent and other factors that can influence the phytochemical composition of plant extracts. Nonetheless, the presence of these metabolites supports *S. occidentalis*'s potential as a source of phytomedicine (Egharevba, 2019).

These phytochemical constituents are biologically active and may contribute to the antiparasitic activity of *S. occidentalis*. Alkaloids and terpenes have been widely reported for their biological and pharmacological activities (Hilal *et al.*, 2024), while tannins and flavonoids have also been associated with antimicrobial activities (Kováč *et al.*, 2023). Saponins have been reported to have several biological activities, including anti-inflammatory effects (Wang *et al.*, 2023). Flavonoids from Nigerian medicinal plants have also been reported to show trypanocidal activity (Achika *et al.*, 2023). These activities suggest that the phytochemicals detected in the present study may contribute to the observed anti-trypanosomal activity of the extract.

The observed antitrypanosomal activity indicates that the methanolic leaf extract of *Senna occidentalis* has potential trypanocidal activity *in vitro*. Reduction in parasite motility is commonly used as an indicator of anti-trypanosomal activity in *in vitro* assays (Alhafiz *et al.*, 2020). The progressive decline in surviving trypanosomes with increasing extract concentration, culminating in complete loss of motility at 5.0 mg/ml, is consistent with previous reports of anti-trypanosomal activity from medicinal plant extracts. Alhafiz *et al.* (2020) reported anti-trypanosomal activity of methanolic extracts from different parts of *S. occidentalis* against *T. brucei*, with the root extract showing the highest inhibition at 500 μ g/ml. More recent studies have also reported *in vitro* anti-trypanosomal activity from Nigerian medicinal plants (Achika *et al.*, 2024). The activity observed in the present study therefore supports further investigation of *S. occidentalis* as a potential source of anti-trypanosomal compounds.

The percentage mortality and LC₅₀ results similarly showed complete loss of motility for both the extract and the reference drug at the highest concentration tested (10 mg/ml) within six hours of incubation. This finding is consistent with previous evidence of anti-trypanosomal activity from *S. occidentalis*. Ibrahim *et al.* (2010) reported complete elimination of *T. brucei* motility within 10 minutes following treatment with 6.66 mg/ml of an ethanol leaf extract. The differences in concentration and incubation time between the two studies may be attributable to differences in extraction solvent, assay conditions and experimental design. Overall, the present findings demonstrate that the methanolic leaf extract of *S. occidentalis* exhibits measurable *in vitro* activity against *T. brucei*.

However, further studies are required to identify its active compounds and assess its *in vivo* effectiveness.

Conclusion

This study has shown that the methanolic leaf extract of *S. occidentalis* possesses anti-trypanosomal activity attributable to its phytochemical constituents, particularly flavonoids, tannins, alkaloids, saponins, and steroids/terpenes. The extract may therefore be considered a potential natural source of anti-trypanosomal agents for therapeutic development. These findings represent a preliminary *in vitro* assessment; further work, including *in vivo* evaluation, is needed to authenticate and standardise the plant's anti-trypanosomal potential accurately, as positive *in vitro* results may not always translate into *in vivo* efficacy.

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REFERENCES

- Achika, J.I., Yusuf, A.J., Ayo, R.G. & Liman, D.U. (2023). Flavonoids from Nigerian indigenous medicinal plants and their bioactivities: a review. *Phytomedicine Plus*, 3:100405.
- Alhafiz, Z.A., Abdulsalami, M.S., Ibrahim, M.A., Bulus, T. & Suleiman, A.B. (2020). Molecular identification of *Trypanosoma brucei* brucei and *in vitro* anti-trypanosomal activity of different parts of methanolic extract of *Senna occidentalis*. *Clinical Phytoscience*, 6:36.
- Alkali, K., Dikwa, K.B., Ajibade, G.A., Magaji, Y. & Abdulhamid, M.B. (2024). Qualitative and quantitative phytochemical screening of aqueous, methanol and hexane leaf extracts of *Senna occidentalis*. *Asian Plant Research Journal*, 12:27-35.
- Badock, E.A., Niang, L., Dramé, A., Nkounkou-Loumpangou, C., Touré, O., Sokhna, O. & Cissé, M. (2024). Phytochemical screening and assays of phenolic compounds in *Senna occidentalis* L. leaf and seed extracts: *Food and Nutrition Sciences*, 15:499-508.
- Bulus, T. & Addau, F.T. (2013). Comparative anti-trypanosomal screening of methanolic extracts of *Khaya senegalensis* and *Moringa oleifera*. *Science World Journal*, 8:1-6.
- Egharevba, H.O. (2019). Chapter 4: Progress in medicinal plant research and traditional medicine practice in Africa. *Chief Editor*, 77.
- Herbert, W.J. & Lumsden, W.H.R. (1976). *Trypanosoma brucei*: a rapid 'matching' method for estimating the host's parasitaemia. *Experimental Parasitology*, 40:427-431.
- Hilal, B., Khan, M.M. & Fariduddin, Q. (2024). Recent advancements in deciphering the therapeutic properties of plant secondary metabolites: phenolics, terpenes, and alkaloids. *Plant Physiology and Biochemistry*, 211:108674.
- Ibrahim, M.A., Aliyu, A.B., Sallau, A.B., Bashir, M., Yunusa, I. & Umar, T.S. (2010). *Senna occidentalis* leaf extract possesses anti-trypanosomal activity and ameliorates the trypanosome-induced anaemia and organ damage. *Pharmacognosy Research*, 2:175.
- Isah, R.T., Mohammed, M.O., Muhammad, A.T., Sahabi, S.M., Umar, Z.U., Mahmud, R.I. & Abubakar, U. (2018). Effects of aqueous leaf extracts of *Senna occidentalis* on rat kidney—*African Journal of Biomedical Research*, 21:225-230.

- Kabila, B., Sidhu, M.C. & Ahluwalia, A.S. (2017). Phytochemical profiling of different *Cassia* species: a review. *International Journal of Pharmaceutical and Biological Archive*, 8:12-20.
- Kováč, J., Slobodníková, L., Trajčiková, E., Rendeková, K., Mučaji, P., Sychrová, A. & Bittner Fialová, S. (2023). Therapeutic potential of flavonoids and tannins in management of oral infectious diseases: a review. *Molecules*, 28:158.
- Mohammed, H. & Alhassan, M. (2017). Effect of crude methanol extract of *Senna occidentalis* on haematological parameters of Wistar rats experimentally infected with *Trypanosoma congolense*—*Journal of Pharmacognosy and Phytochemistry*, 6:1217-1224.
- Nekoei, S., Khamesipour, F., Habtemariam, S., de Souza, W., Mohammadi Pour, P. & Hosseini, S.R. (2022). The anti-trypanosoma activities of medicinal plants: a systematic review of the literature. *Veterinary Medicine and Science*, 8:2738-2772.
- Odeniran, P.O., Ademola, I.O., Macleod, E.T. & Welburn, S.C. (2018). Bovine and small ruminant African animal trypanosomiasis in Nigeria – a review. *Veterinary Parasitology: Regional Studies and Reports*, 13:5-13.
- Rascón-García, K., Martínez-López, B., Cecchi, G., Scoglio, C., Matovu, E. & Muhanguzi, D. (2023). Prevalence of African animal trypanosomiasis among livestock and domestic animals in Uganda: a systematic review and meta-regression analysis from 1980 to 2022. *Scientific Reports*, 13:20337.
- Ungogo, M.A. & de Koning, H.P. (2024). Drug resistance in animal trypanosomiasis: epidemiology, mechanisms and control strategies. *International Journal for Parasitology: Drugs and Drug Resistance*, 25:100533.
- Veronique, J.E. & Gabriel, N.M. (2013). Subchronic toxicity of the beverage made from *Cassia occidentalis* seeds in mice—*International Journal of Nutrition and Food Sciences*, 2:237-242.
- Wang, X., Ma, Y., Xu, Q., Shikov, A.N., Pozharitskaya, O.N., Flisyuk, E.V., Liu, M., Li, H., Vargas-Murga, L. & Duez, P. (2023). Flavonoids and saponins: what have we got or missed? *Phytomedicine*, 109:154580.
- World Health Organization (WHO) (2019). *Traditional Healers Broaden Health Care in Ghana*. Geneva: WHO..