FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 10 No. 9, May, 2026, pp 274 – 277
DOI: https://doi.org/10.33003/fjs-2026-1009-4994

Phytochemical Profiling and in Vitro Antitrypanosomal Efficacy of the Aqueous Leaf Extract of *Combretum glutinosum* (L.) Against *Trypanosoma brucei*

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Received: 16 May 2026
Accepted: 04 June 2026
Published: 29 June 2026

ABSTRACT

African trypanosomiasis, a severely neglected tropical disease, is persistently plagued by a limited arsenal of therapeutic options that are frequently associated with high toxicity and emerging resistance. This study systematically investigated the phytochemical composition and the in vitro antitrypanosomal potential of an aqueous leaf extract of *Combretum glutinosum* against *Trypanosoma brucei*. Fresh leaves were collected, shade-dried, and extracted via maceration in distilled water. Qualitative phytochemical analysis confirmed the presence of diverse secondary metabolites, including tannins, saponins, phenols, alkaloids, carbohydrates, cardiac glycosides, flavonoids, terpenoids, and steroids, while anthraquinones were absent. The antitrypanosomal activity was rigorously evaluated using a 96-well microtiter plate assay across a broad concentration range of 31.25–500 mg/mL. Parasite motility was monitored microscopically at 5-minute intervals over a 60-minute incubation period. The aqueous extract demonstrated a pronounced, concentration-dependent trypanocidal effect. Complete cessation of parasite motility was achieved at 45 minutes for the highest concentration (500 mg/mL), at 55 minutes for 250 mg/mL, and at 60 minutes for 125 mg/mL. Lower concentrations (62.5 and 31.25 mg/mL) exhibited progressively reduced efficacy. Assay validity was confirmed using diminazene diaceturate as the positive control and phosphate-buffered saline as the negative control. These compelling results indicate that *C. glutinosum* harbours bioactive constituents with promising antitrypanosomal activity, thereby scientifically validating its ethnomedicinal use and underscoring its potential as a valuable source of novel trypanocidal lead compounds for future drug development.

Keywords: Aqueous extract, Antitrypanosomal, *Combretum glutinosum*, Phytochemicals, *Trypanosoma brucei*

INTRODUCTION

Trypanosomiasis, encompassing Human African Trypanosomiasis (HAT, commonly known as sleeping sickness) and animal trypanosomiasis (Nagana), remain formidable vector-borne diseases with profound public health and economic impacts across sub-Saharan Africa (Brahma *et al.*, 2025). The primary causative agents, *Trypanosoma brucei* and related species, induce complex pathophysiological changes in the host, leading to a spectrum of debilitating symptoms such as intermittent fever**,** progressive weight loss, muscle weakness, anemia, and general body emaciation, and, in humans, severe neurological disruption that is invariably fatal if left untreated (Ponte-Sucre, 2016). The current landscape of pharmacotherapy, which includes drugs such as suramin, pentamidine, melarsoprol, and the newer oral agent fexinidazole, is heavily hampered by significant challenges, including severe systemic toxicity, complex and prolonged administration routes, and the alarming emergence of drug-resistant parasite strains (Barrett, 2025). The historical classification of these diseases as 'neglected' has severely stifled pharmaceutical investment and drug development, creating an urgent and critical need for the discovery of safer, more effective, and accessible chemotherapeutic agents (Weng *et al.*, 2018).

Medicinal plants represent a vital and historically rich reservoir for novel drug discovery, particularly in regions where approximately 80% of the population relies on traditional herbal medicine for primary healthcare needs (Napagoda and Wijesundara, 2022). *Combretum glutinosum*, a prominent member of the Combretaceae family, is extensively utilized in traditional medicine systems across West Africa for the treatment of various ailments, including malaria, wounds, and diverse microbial infections (Garba *et al.*, 2025). While previous scientific investigations have documented its significant antimicrobial, antioxidant, and anti-inflammatory properties (Ibrahim *et al.*, 2024), its specific pharmacological activity against African trypanosomes remains inadequately explored in contemporary literature. Therefore, this study aims to evaluate the phytochemical composition and assess the in vitro antitrypanosomal activity of the aqueous leaf extract of *Combretum glutinosum* (L.) against *Trypanosoma brucei*. The objectives of this paper are to qualitatively determine the phytochemical constituents present in the aqueous leaf extract of *Combretum glutinosum*. To evaluate the in vitro antitrypanosomal effect of the aqueous leaf extract of *Combretum glutinosum* on the motility of *Trypanosoma brucei* at varying concentrations and time intervals and to comprehensively evaluate the phytochemical constituents and the in vitro antitrypanosomal efficacy of the aqueous leaf extract of *C. glutinosum* against *T. brucei*, providing scientific evidence to support its ethnomedicinal applications. The

aqueous leaf extract of *C. glutinosum* does not possess significant in vitro antitrypanosomal activity against *T. brucei*.

MATERIALS AND METHODS

Plant Material Collection and Authentication

Fresh leaves of *C. glutinosum* were harvested from the natural vegetation in Kufena, Zaria, Kaduna State, Nigeria (Latitude 11°51′2″ N, Longitude 7°39′22″ E). Botanical identification and authentication were rigorously confirmed by a herbarium curator, and a voucher specimen (ABUH07083) was given at the Herbarium Unit of the Department of Botany, Ahmadu Bello University, Zaria, for future reference.

Preparation of Aqueous Extract

The collected fresh leaves were meticulously washed with clean water to remove dust and debris, and then air-dried at ambient room temperature under shade for two weeks to prevent the thermal degradation of heat-sensitive active compounds. The dried leaves were pulverized into a fine powder using a mechanical grinder. The extraction process involved subjecting the powdered plant material to maceration in distilled water for a period of 72 hours, with intermittent manual shaking to ensure optimal extraction. The resultant mixture was filtered, and the filtrate was concentrated to dryness using a rotary evaporator set at 80°C to yield a dry, crude aqueous extract. The extract was stored in a desiccator until required for further use (Harborne, 1998).

Qualitative Phytochemical Screening

Standard qualitative phytochemical protocols were strictly employed to detect the presence of major classes of secondary metabolites in the aqueous extract (Brain and Turner, 2005). These analytical tests included Mayer's reagent for alkaloids, the Shinoda test for flavonoids, the ferric chloride test for tannins, the frothing test for saponins, the lead acetate test for phenols, the Liebermann–Burchard test for terpenoids, Salkowski's test for steroids, the Keller–Kiliani test for cardiac glycosides, Molisch's test for carbohydrates, and Borntrager's test for anthraquinones (Edeoga *et al*., 2005).

Parasite Isolation and Cultivation

The *T. brucei* strain used in this study was acquired from the Nigerian Institute for Trypanosomiasis Research (NITR), located in Kaduna, Nigeria. The parasites were maintained in vivo through serial passage in healthy albino rats. The parasitaemia levels in the infected host blood were quantified daily using the standard rapid matching technique as described by Takeet *et al*. (2012).

In Vitro Antitrypanosomal Assay

The antitrypanosomal activity of the extract was systematically assessed using a modified 96-well microtiter plate method as described by Cross and Manning (1973). Briefly, 20 µL of infected rat blood (containing approximately 10^6 parasites/mL) was carefully incubated with 10 µL of the aqueous extract to achieve final test concentrations of 31.25, 62.5, 125, 250, and 500 mg/mL. Diminazene diaceturate (10 µg/mL) and plain phosphate-buffered saline (PBS) served as the standard positive and negative controls, respectively. Parasite motility was meticulously observed under a light microscope at 400× magnification at 5-minute intervals over a comprehensive 60-minute incubation period. The motility of the parasites was scored semi-quantitatively as follows: very active (++++), slightly weak (++), weak (+), or no motility/dead (–).

Statistical Analysis

All experimental data are expressed as the mean ± standard deviation of triplicate determinations. Statistical significance was determined using one-way analysis of variance (ANOVA), and a p-value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical Constituents

The qualitative phytochemical screening of the aqueous leaf extract of *C. glutinosum* revealed a rich and complex profile of secondary metabolites. The chemical tests confirmed the presence of tannins, saponins, phenols, alkaloids, carbohydrates, cardiac glycosides, flavonoids, terpenoids, and steroids. However, anthraquinones were not detected in the extract (Table 1).

Table 1: Phytochemical Composition of Aqueous Leaf Extract of *Combretum glutinosum*

Compound	Test	Inference
Tannins	Ferric chloride	+
Saponins	Frothing	+
Phenols	Lead acetate	+
Alkaloids	Mayer's	+
Carbohydrates	Molisch's	+
Cardiac Glycosides	Keller-Kiliani	+
Flavonoids	Sodium hydroxide	+
Terpenoids	Liebermann-Burchard	+
Steroids	Salkowski	+
Anthraquinones	Borntrager's	-

+ = Present; - = Absent.

In Vitro Antitrypanosomal Activity

The aqueous extract exhibited a pronounced, concentration-dependent suppression of *T. brucei* motility in vitro (Table 2). At the highest concentration tested (500 mg/mL), complete immobilisation of all parasites was achieved within 45 minutes of incubation. Concentrations of 250 mg/mL and 125 mg/mL induced total motility cessation at 55 minutes. The lower concentrations of 62.5 mg/mL and 31.25 mg/mL showed modest and minimal effects, respectively, with parasites remaining largely motile throughout the observation window. The positive control (diminazene diaceturate) caused rapid parasite immobilisation, whereas the negative control (PBS) showed no antitrypanosomal activity, with parasites remaining very active.

Table 2: Motility Response of *Trypanosoma brucei* to Aqueous Extract of *Combretum glutinosum* Over 60 Minutes

Concentration (mg/mL)	5	10	15	20	25	30	35	40	45	50	55	60
Control (+)	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Control (-)	-	-	-	-	-	-	-	-	-	-	-	-
500	++++	++++	+++	+++	+++	++	++	++	-	-	-	-
250	++++	++++	++++	++++	+++	+++	+++	++	++	++	-	-
125	++++	++++	++++	++++	++++	++++	+++	+++	+++	++	-	-
62.5	++++	++++	++++	++++	++++	++++	++++	++++	+++	+++	-	-
31.25	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	+++

++++ = Very active; +++ = Active; ++ = Slightly weak; + = Weak; - = No motility/Dead.

Discussion

The comprehensive phytochemical screening conducted in this study corroborates the rich diversity of bioactive compounds present in *C. glutinosum*, closely aligning with its widely documented ethnopharmacological uses across Africa (Mohammed *et al*., (2022); Sweilam *et al.*, 2023). The detected constituents particularly the alkaloids, flavonoids, tannins, and terpenoids are widely implicated in conferring potent antiprotozoal activities (Gulsen *et al*., 2025; Kontagora and Ibrahim, (2025). These diverse classes of compounds can exert their trypanocidal effects through multiple complex mechanisms, which may include the disruption of parasite cell membrane integrity, the competitive inhibition of essential metabolic enzymes, the chelation of vital metal ions, and the induction of severe oxidative stress within the parasite (Isah *et al.*, 2018).

The clearly observed concentration-dependent antitrypanosomal activity of the extract is highly consistent with the typical pharmacological behaviour exhibited by many plant-derived crude extracts (Martín-Martín *et al.*, 2026). The potent activity recorded at 500 mg/mL, which resulted in complete motility loss within 45 minutes, strongly suggests the presence of synergistic interactions among the constituent phytochemicals. However, the markedly reduced efficacy observed at lower concentrations (≤62.5 mg/mL) highlights a critical pharmacological threshold necessary for effective parasite suppression. This specific pattern of activity underscores the absolute necessity for future studies aimed at bioassay-guided fractionation to isolate, purify, and structurally characterize the specific compound(s) responsible for the observed activity. This is in line with the findings of Konwar and Shah (2026) who studied the Anthelmintic potential of methanolic leaf extract of *Sarcochlamys pulcherrima* (Roxb.) Gaudich. against *Raillietina echinobothrida* (Megnin) and discovered that potent activity resulted in complete motility loss within some minutes. Recent comprehensive reviews by El-Saadony *et al.* (2025) strongly emphasize that such isolation is a crucial and indispensable step in transforming promising crude plant extracts into viable, standardized lead compounds with highly optimized potency and safety profiles.

While the in vitro results presented herein are highly promising, they represent only an initial step in the complex drug discovery pipeline. The relatively high effective concentration (500 mg/mL) required compared to the standard pharmaceutical drug control clearly indicates that further purification is essential to significantly improve specific activity. Furthermore, the potential cytotoxicity of the crude extract against mammalian host cells was not assessed in this current study and undoubtedly warrants thorough investigation (Ahmad Suhaimi *et al*., 2026). Subsequent research efforts should therefore focus intensively on conducting robust in vivo efficacy studies using appropriate animal models, performing detailed mechanistic investigations at the molecular level, and carrying out comprehensive toxicological evaluations to fully and accurately appraise the therapeutic potential of *C. glutinosum* derivatives against African trypanosomiasis.

CONCLUSION

The aqueous leaf extract of *Combretum glutinosum* contains several bioactive compounds, including tannins, saponins, phenols, alkaloids, flavonoids, terpenoids, steroids, cardiac glycosides, and carbohydrates (all present (+), while anthraquinones were absent (-). This indicates a rich phytochemical profile that may contribute to its biological activity.

The extract exhibited a concentration- and time-dependent reduction in parasite motility, with the highest concentration (500 mg/mL) causing complete loss of motility (-) at 45–60 minutes, while lower concentrations such as 31.25 mg/mL maintained high motility (++++ to +++) even at 60 minutes, demonstrating reduced efficacy at lower doses.

Therefore, further studies should be conducted to isolate, characterize, and quantify the specific bioactive compounds responsible for the observed biological activities, as well as to evaluate the extract through in vivo studies and dose optimization to validate its efficacy and safety as a potential treatment for trypanosomiasis.

CONFLICT OF INTEREST

There is no conflict of interest.

FUNDING FOR THE WORK

This research was not financially supported by any agencies.

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