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Comparative Phytochemical Profiling and In Vitro Antitrypanosomal Efficacy of Methanolic Leaf Extracts of *Ocimum sanctum* L. and *Grewia mollis* Against *Trypanosoma brucei*

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Abstract

African trypanosomiasis, a profoundly neglected tropical disease caused by the protozoan parasite of the genus *Trypanosoma*, persists as a critical public health and economic challenge across sub-Saharan Africa. Despite recent progress towards disease elimination, significant concerns regarding emerging drug resistance and the severe limitations of current chemotherapies remain. This comparative study systematically examined the phytochemical composition and the in vitro antitrypanosomal effects of methanolic leaf extracts derived from *Ocimum sanctum* L. (holy basil) and *Grewia mollis* (Malvaceae), two plants with established and widespread ethnomedicinal use in Nigeria. Fresh leaves were botanically authenticated, shade-dried, powdered, and subsequently extracted using methanol. Qualitative phytochemical screening successfully identified shared bioactive constituents including flavonoids, terpenoids, tannins, saponins, steroids, phenolic compounds, cardiac glycosides, and carbohydrates. Notably, alkaloids were detected only in *G. mollis*, while anthraquinones were absent in both extracts. Motility inhibition assays conducted against bloodstream forms of *T. brucei brucei* across a concentration range of 31.25–500 µg/mL revealed pronounced concentration- and time-dependent trypanocidal activity for both extracts. The G. mollis extract achieved faster complete parasite immobilisation (30 minutes at 250–500 µg/mL; 45 minutes at lower doses) than *O. sanctum* (40 minutes at higher doses, with slower progression at lower concentrations). Diminazene aceturate (positive control) caused immediate parasite death, while the phosphate-buffered saline (negative control) sustained vigorous motility throughout the observation period. These compelling results scientifically validate the traditional medicinal applications of both plants and attribute their antitrypanosomal

effects to synergistic phytochemical interactions, likely involving membrane disruption and the induction of severe oxidative stress. Furthermore, the findings highlight G. mollis as a particularly promising candidate for drug discovery amid growing resistance threats, underscoring the necessity for future bioassay-guided fractionation and comprehensive in vivo efficacy studies.

Introduction

Human African trypanosomiasis (HAT, commonly known as sleeping sickness), primarily caused by *Trypanosoma brucei gambiense* and *T. b. rhodesiense*, and animal African trypanosomiasis (AAT, or Nagana), involving *T. brucei* and closely related species, remain major and highly neglected tropical diseases endemic to sub-Saharan Africa (Lutumba et al., 2024). While reported HAT cases have declined dramatically, achieving a 98% reduction from 1999 to 2024 for the gambiense form, the disease is far from eradicated. In 2024, 546 new cases were recorded, predominantly in West and central Africa (where *T. b. gambiense* accounts for over 94% of cases), with the rhodesiense form persisting in eastern and southern regions (Rinaldi et al., 2026).

Concurrently, AAT continues to cause substantial and devastating livestock losses, significantly exacerbating rural poverty and threatening food security across the continent (Majamanda et al., 2026). The current landscape of chemotherapy options, which includes older drugs such as pentamidine, suramin, melarsoprol, and eflornithine/nifurtimox combination therapies, as well as the newer oral agent fexinidazole, is fraught with severe challenges (Hidalgo et al., 2021). These include high systemic toxicity, complex administration protocols, prohibitive costs, limited patient access, and the alarming, documented emergence of drug-resistant parasite strains. This precarious situation underscores an urgent need for robust surveillance for resistant strains and the rapid development of novel therapeutics.

Medicinal plants have long provided a rich and diverse reservoir of bioactive secondary metabolites that serve as novel structural leads for drug discovery, often exhibiting multi-target and synergistic pharmacological effects (Latif & Nawaz, 2026). In Nigeria, traditional healers extensively utilize various plant leaf extracts for the management of parasitic infections, including trypanosomiasis (Nafiu et al., 2021; Chukwudi et al., 2026). *Ocimum sanctum* L. (family Lamiaceae, commonly known as holy basil or Tulsi) is well-documented for its potent antioxidant, antimicrobial, and antiprotozoal properties. These activities are largely attributed to its rich content of eugenol, ursolic acid, flavonoids, terpenoids, and tannins (Nafiu et al., 2021; Maphetu et al., 2026). Furthermore, the closely related species *O. gratissimum* has demonstrated strong in vitro antitrypanosomal activity, with methanolic and ethanolic extracts exhibiting IC50 values of approximately 1-2 μg/mL against T. brucei (Nafiu et al., 2021). This potent activity has been directly linked to both its essential oils and non-volatile constituents, which are believed to disrupt parasite cell membranes (Manjarrez-Quintero et al., 2026). *Grewia mollis* (family Malvaceae), traditionally used for treating fevers and various microbial infections, is known to contain alkaloids, flavonoids, tannins, saponins, and terpenoids. While it possesses recognized antimicrobial potential, specific data regarding its antitrypanosomal efficacy remain limited but highly promising within the broader context of African medicinal plant reviews (Dev et al., 2019; Upahi et al., 2026).

This study aims to comparatively evaluate the qualitative phytochemical composition and in vitro antitrypanosomal activity of methanolic leaf extracts of *Ocimum sanctum* [L.] and *Grewia mollis* against *Trypanosoma brucei*, with particular emphasis on their effects on parasite motility at different concentrations and time intervals.

Materials and Methods

Plant Material Collection and Authentication

Fresh leaves of *Ocimum sanctum* were collected from the premises of the Ahmadu Bello University Teaching Hospital, Zaria, Kaduna State, Nigeria

(Latitude 11.1113° N, Longitude 7.7227° E) in June 2025. The plant material was assigned the voucher number ABUTH07028. Concurrently, fresh leaves of *Grewia mollis* were obtained from the natural vegetation in the environs of Zaria, and assigned the voucher number ABUH09038. Both plant specimens were formally identified and authenticated by a senior taxonomist at the Herbarium of the Department of Botany, Ahmadu Bello University, Zaria, where the voucher specimens were deposited for future reference.

Preparation and Extraction

The freshly harvested leaves of both plant species were thoroughly washed with clean distilled water to remove surface contaminants. They were then shade-dried at room temperature for a period of two weeks to prevent the thermal degradation of sensitive phytochemicals (Nafiu et al., 2021; Kontagora & Ibrahim, 2025). Once completely dried, the leaves were pulverized into a coarse powder using a mechanical grinder. For the extraction process, 119 g each of the powdered *O. sanctum* leaves and *G. mollis* were macerated in 800 mL of 80% methanol. Both extractions were carried out for 72 hours with intermittent manual shaking. The resulting mixtures were filtered through Whatman No. 1 filter paper. The filtrates were then concentrated under reduced pressure at 50°C using a BÜCHI R-110 rotary evaporator. The resulting crude methanolic extracts were stored at 4°C in sterile, airtight containers until required for further analysis.

Phytochemical Screening

Comprehensive qualitative phytochemical analyses were conducted on both methanolic extracts using established standard protocols (Nafiu et al., 2021; Kontagora and Ibrahim, 2025; Möwes et al., 2025). The tests performed aimed to identify the presence of major secondary metabolite classes, including alkaloids (using Dragendorff's, Mayer's, and Wagner's reagents), tannins (ferric chloride and bromine water tests), saponins (frothing test), flavonoids (Shinoda test and alkaline reagent test), cardiac glycosides (Keller-Kiliani test), terpenoids (Liebermann-Burchard test), steroids (Salkowski test), carbohydrates (Molisch test), phenols (lead acetate test), and anthraquinones (Borntrager's test).

Parasite Maintenance and In Vitro Antitrypanosomal Assay

The *Trypanosoma brucei* strain utilized in this study was sourced from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna. The parasite was continuously maintained in vivo in healthy albino rats and mice through regular serial passage. Parasitaemia levels were rigorously monitored daily by examining wet mounts of tail blood under a microscope. Highly infected blood, exhibiting moderate to high parasitaemia, was collected via cardiac puncture into EDTA-coated tubes to prevent coagulation. For the in vitro assay, the crude extracts were initially dissolved in phosphate-buffered saline (PBS, pH 7.2) and subsequently serially diluted to achieve final test concentrations of 500, 250, 125, 62.5, and 31.25 μg/mL. In sterile 96-well microtiter plates or test tubes, 0.5 mL of the infected blood was mixed in a 1:1 ratio with the respective extract dilutions. The mixtures were then incubated at a controlled room temperature of 25 ± 2°C. Diminazene aceturate (7.5 mg/mL) and plain PBS were used as the standard positive and negative controls, respectively. Parasite motility, serving as a reliable indicator of viability, was assessed microscopically at 400× magnification at 5-minute intervals over a total duration of 60 minutes. Motility was scored semi-quantitatively as: ++++ (very active), +++ (active), ++ (slightly weak), + (weak), or – (dead/completely immobilised). All experimental assays were performed in triplicate to ensure data reliability.

Data Analysis

The collected data regarding parasite motility over time for each tested concentration were analysed descriptively. The specific time required to achieve complete immobilisation (a score of '–') of all parasites was carefully recorded and compared between the two plant extracts and across the different concentration gradients.

Results

Phytochemical Constituents

The qualitative phytochemical screening revealed that both methanolic extracts possessed a rich and

diverse array of secondary metabolites (Table 1). Both plants shared several classes of bioactive compounds known to support antiparasitic synergy, including cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids, and terpenoids. Notably, alkaloids were detected exclusively in the *G. mollis* extract, while anthraquinones were completely absent in both plant extracts.

In Vitro Antitrypanosomal Activity

Both methanolic extracts exhibited potent, concentration- and time-dependent motility inhibition against *T. brucei* in vitro (Table 2). The negative control (PBS) sustained very active (++++) parasite motility throughout the entire 60-minute observation period, whereas the positive control (diminazene aceturate) achieved immediate and complete parasite death (–). At the higher test concentrations (250–500 μg/mL), both extracts induced a rapid decline in parasite motility. However, *G. mollis* demonstrated superior rapidity in its trypanocidal action, achieving complete immobilisation of all parasites within 35 minutes at concentrations of 250–500 μg/mL, and within 40 minutes at the lower concentrations of 31.25–125 μg/mL. In contrast, the *O. sanctum* extract required 40 minutes to achieve complete immobilisation at the higher doses, and exhibited a more progressive but noticeably slower effect at the lower concentrations (for instance, weak motility was still observable at 60 minutes for the 31.25 μg/mL concentration).

Discussion

The pronounced concentration- and time-dependent antitrypanosomal activity observed in this study aligns perfectly with recent comprehensive reviews of African medicinal plants, which frequently report that methanolic extracts exhibit potent parasite inhibition driven by a diverse array of secondary metabolites (Nafiu et al., 2021; Nagori et al., 2025). The rich suite of shared phytochemicals identified in both extracts including flavonoids, terpenoids, tannins, saponins, phenols, and cardiac glycosides highly likely contributes to the observed trypanocidal effects through complex, multifaceted, and synergistic mechanisms (Nafiu et al., 2021; Agrawal et al., 2025; Kontagora & Ibrahim, 2025). Specifically, saponins and terpenoids are well-known to intercalate with and severely disrupt the structural integrity of parasite cell membranes, leading to increased permeability and catastrophic cell leakage. Furthermore, flavonoids and phenolic compounds can act as potent pro-oxidants within the specific cellular environment of trypanosomes (which notably lack robust, mammalian-like antioxidant defense systems), thereby generating lethal levels of reactive oxygen species (ROS). Additionally, tannins possess the ability to complex with and precipitate vital surface proteins and metabolic enzymes, while cardiac glycosides are known to potently interfere with essential ion transport mechanisms, such as the Na+/K+-ATPase pump.

Table 1: Comparative Qualitative Phytochemical Constituents of *O. sanctum* and *G. mollis*

Phytoconstituents	Test	*O. sanctum*	*G. mollis*
Alkaloids	Dragendorff test	-	+
Cardiac Glycosides	Keller-Kiliani test	+	+
Saponins	Frothing test	+	+
Phenolic compounds	Lead acetate test	+	+
Tannins	Ferric Chloride test	+	+
Steroids	Salkowski test	+	+
Carbohydrates	Molisch test	+	+
Flavonoids	Shinoda test	+	+
Terpenoids	Liebermann-Burchard test	+	+
Anthraquinones	Borntrager's test	-	-

Note. + = Present; - = Absent.

Table 2: Comparative Motility Scores of *T. brucei* Over Time (Selected Key Time Points)

Conc. (µg/mL)	Plant	5	10	15	20	25	30	35	40	45	50	55	60
500	*O. sanctum*	++	++	++	++	+	+	+	-	-	-	-	-
500	*G. mollis*	++	++	+	+	+	+	-	-	-	-	-	-
250	*O. sanctum*	+++	+++	+++	+++	++	++	++	+	-	-	-	-
250	*G. mollis*	++	++	+	+	+	+	-	-	-	-	-	-
125	*O. sanctum*	+++	+++	+++	+++	+++	+++	++	++	+	+	-	-
125	*G. mollis*	+++	+++	+++	+++	++	++	+	-	-	-	-	-
62.5	*O. sanctum*	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	+
62.5	*G. mollis*	++++	++++	+++	+++	++	++	+	+	-	-	-	-
31.25	*O. sanctum*	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	+
31.25	*G. mollis*	++++	++++	+++	+++	++	+	+	-	-	-	-	-
Control (-)	PBS	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Control (+)	Diminazene	-	-	-	-	-	-	-	-	-	-	-	-

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

Similar reports were obtained by Omole et al. (2025) who studied Cardiac glycosides: looking beyond heart failure and atrial fibrillation and discovered that cardiac glycosides are known to potently interfere with essential ion transport mechanisms. The detection of alkaloids uniquely within the *G. mollis* extract is a finding of significant pharmacological interest. Similar findings were observed by Eduardo & Vásquez-Senador (2026) who studied Minthostachys mollis: Chemical composition, biological activities and potential applications in a systematic and bibliometric review and realized that alkaloids are uniquely detyected. Alkaloids are renowned for their potent biological activities and may significantly enhance the overall trypanocidal potency of the extract by specifically targeting and disrupting parasite nucleic acid synthesis or inhibiting critical metabolic enzymes. This distinct phytochemical difference provides a highly plausible biochemical explanation for the faster rate of parasite immobilisation consistently observed with the G. mollis extract compared to *O. sanctum* (Nafiu et al., 2021; García et al., 2025; Kontagora & Ibrahim, 2025). The robust activity recorded for *O. sanctum* closely mirrors the findings reported for related Ocimum species. For instance, methanolic and ethanolic extracts of *O. gratissimum* have been shown to possess strong in vitro activity (with IC50 values of ~1-2 µg/mL) against *T. brucei*, an effect primarily attributed to the presence of eugenol-like compounds that cause severe damage to both the parasite membrane and its kinetoplast (Nafiu et al., 2021; Ojewumi et al., 2025). The synergistic interactions occurring among these diverse classes of phytochemicals undoubtedly amplify their overall therapeutic efficacy, a phenomenon that is highly consistent with broader, systematic screenings of Nigerian medicinal plants (Nafiu et al., 2021; Kontagora & Ibrahim, 2025; Raheem et al., 2026). The demonstrably superior rapidity of action exhibited by G. mollis strongly suggests either a more efficient extraction of bioactive principles or an inherently higher trypanocidal potency of its specific chemical constituents, thereby providing robust scientific support for its distinct ethnomedicinal applications.

Conclusion

This study shows that methanolic leaf extracts of *Grewia mollis and Ocimum sanctum* exhibit concentration dependent antitrypanosomal activity. *Grewia mollis* demonstrated greater efficacy, likely associated with alkaloids detected in its phytochemical profile, suggesting its potential for further investigation as an antitrypanosomal agent.

Recommendations

Further research should involve in vivo validation and toxicity studies of *Grewia mollis* to establish its potential as a more effective antitrypanosomal agent and to determine safe therapeutic dosage levels.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: study design: RIA; data collection: USB and MH; analysis and interpretation of results: NMI; draft manuscript preparation: MM and ASG. All authors reviewed the results and approved the final version of the manuscript.

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