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Enhanced Trypanocidal Activity of Green-Synthesized Silver Nanoparticles from *Gliricidia sepium* Leaf Extract against *Trypanosoma brucei brucei*: an *in vitro* Study

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Abstract

Trypanosomiasis, caused by *Trypanosoma spp*., remains a major health and economic challenge, particularly in sub-Saharan Africa. Current treatments are limited by toxicity, high costs, and emerging drug resistance, necessitating the search for alternative therapeutic options. This study investigates the trypanocidal activity of *Gliricidia sepium* leaf extracts and its green synthesized silver nanoparticles (G.s-AgNPs) against *Trypanosoma brucei brucei*. Ethanolic extraction of *G. sepium* leaves was performed, and phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, terpenoids, saponins, and glycosides. Green synthesis of silver nanoparticles was conducted using aqueous *G. sepium* extracts and characterized by UV-Vis spectroscopy, ATR-FTIR, SEM, and XRD analyses. The in vitro trypanocidal activity of the crude extract and G.s-AgNPs was assessed using the drug incubation survival assay. Results demonstrated a concentration-dependent trypanocidal effect, with G.s-AgNPs exhibiting superior efficacy compared to the crude extract (G.s-AgNPs showed 95% reduction at 10 mg/mL, outperforming crude extract). The nanoparticles significantly reduced trypanosome survival rates at lower concentrations, indicating enhanced bioactivity likely due to improved cellular interaction and uptake. These findings suggest that *G. sepium* and its silver nanoparticles hold promise as alternative trypanocidal agents. This study highlights the potential of integrating medicinal plant extracts with nanotechnology to enhance trypanocidal activity. Further *in vivo* validation is needed and toxicity assessments are also necessary to validate their safety and therapeutic potential.

Keywords: *Gliricidia sepium,* Trypanosomiasis, Green Synthesis, Silver Nanoparticles, Antitrypanosomal Activity, Medicinal Plants.

Introduction

Trypanosomiasis, a disease caused by protozoan parasites of the genus Trypanosoma, poses a major health and economic burden in Africa, Asia, and Latin America, affecting both humans and livestock (Hotez and Kamath, 2009). In Africa, human African trypanosomiasis (HAT), also known as African sleeping sickness, is a life-threatening disease caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, transmitted by the tsetse fly (Glossina spp.) (Sutherland et al., 2015). While *T. brucei rhodesiense* causes East African HAT, T. brucei gambiense is responsible for West African HAT, both presenting with hemolymphatic and meningo-encephalitic stages (Büscher et al., 2017). Despite being classified as a neglected tropical disease, HAT remains a significant public health threat in rural Africa (Stich et al., 2002). Similarly, African animal trypanosomiasis (AAT) represents a major constraint to livestock production in tsetse fly-infested regions of sub-Saharan Africa, leading to an estimated 3 million livestock deaths annually and significant economic losses (Abenga et al., 2002; Imam et al., 2021). The disease manifests with fever, anemia, cachexia, edema, and reproductive disorders, often resulting in mortality if left untreated (FAO, 2002). Current chemotherapy for trypanosomiasis relies on a limited number of drugs, many of which are associated with toxicity, high costs, and the emergence of drug-resistant Trypanosoma strains (Venturelli et al., 2022).

Consequently, there is an urgent need for alternative, safer, and more effective trypanocidal agents. Medicinal plants have long played a significant role in traditional medicine for the treatment of various diseases (Fallah-Hoseini *et al*., 2006). Many plant species have been identified with trypanocidal properties, suggesting the potential to develop novel treatments from natural sources (Asuzu and Chineme, 1990; Freiburghaus *et al*., 1996; Nok *et al*., 1996; Atawodi *et al*., 2003; Ogbunugafor *et al*., 2007; Nwodo *et al*., 2007; Maikai *et al*., 2008; Abedo *et al*., 2013). Among these, *Gliricidia sepium*, a member of the Fabaceae family, has shown promise as a multipurpose medicinal plant with potential trypanocidal activity. Although direct investigations into its antitrypanosomal efficacy are limited, a study by Adekunle and Akinyemi (2004) reported inhibitory effects of *G. sepium* extracts on Trypanosoma species, indicating its potential as a natural source of antiparasitic agents. Additionally, Akinmoladun *et al*. (2020) observed moderate in vitro antitrypanosomal activity in crude extracts of *G. sepium*, supporting its traditional use and highlighting the need for further research into its bioactive compounds and mechanisms of action.

According to Abdelhameed *et al* (2023), in their review titled "An Overview On *Gliricidia Sepium* In The Pharmaceutical Aspect: A Review Article" reported Many medicinal uses for *Gliricidia sepium* were discovered over time, including anti-microbial activity, antibacterial activity, cytotoxic activity, antioxidant activity, anti-inflammatory activity, thrombolytic, wound healing, anti-sickling activity, mosquitocidal activity, and anthelmintic activity (Abdelhameed *et al.,* 2023). Several studies have also explored the bioactivity of *G. sepium* more broadly. Its extracts have demonstrated antimicrobial (Adegbite *et al*., 2013), antiinflammatory (Dapar *et al*., 2007), antioxidant (Kumar *et al*., 2012), and anthelmintic (Néstor *et al*., 2020) activities, suggesting that the plant contains a range of phytochemicals with therapeutic potential. Phytochemical analyses have identified flavonoids, tannins, saponins, and phenolic compounds as the key constituents responsible for these effects (Alonso-Castro *et al*., 2011; Ezeonu *et al*., 2022).

We hypothesize that nano-formulation enhances bioavailability and cellular uptake of phytochemicals, potentially amplifying the trypanocidal effects of *G. sepium* extracts by improving solubility, stability, and targeted delivery of the active constituents.

However, concerns about drug resistance and toxicity have prompted investigations into combination therapies. For example, combining secnidazole with Diminazene aceturate has shown enhanced therapeutic efficacy compared to monotherapy in canine models (Eke *et al*., 2020). While specific studies on *Gliricidia sepium* extracts against *T. brucei* are limited, related research indicates potential antiparasitic properties. Ethanol extracts from *G. sepium* have demonstrated anthelmintic activity in vitro, suggesting possible applications against parasitic infections (Néstor *et al*., 2020).

This study investigates the trypanocidal activity of leaf extracts from *Gliricidia sepium* as well as its mediated silver

nanoparticles to evaluate its potential as an alternative treatment for trypanosomiasis. While *Gliricidia sepium* (Figurre 1) is well-documented for its use as a forage plant and in agroforestry (Wiersum and Nitis 1992; Heuzé and Tran, 2015), its potential as a trypanocidal agent has not been fully explored. By assessing it is *in vitro* activity against *Trypansoma brucei brucei*, this research aims to provide new insights into its pharmacological properties and contribute to the development of alternative trypanocidal treatments. The findings will expand the knowledge of medicinal plant-based therapeutics and may offer a cost-effective and accessible option for combating trypanosomiasis, particularly in endemic regions.

Figure 1: *Gliricidia sepium* image adopted from West African Plants: A Photo Guide

MATERIALS AND METHODS

Collection and identification of plant extract

The *Gliricidia sepium* plant was collected at Kaduna North LGA, Kaduna, Nigeria. Identified and authenticated by a taxonomist at the Department of Biological Science Herbarium, Ahmadu Bello University, Zaria, Kaduna, Nigeria, with voucher numbers V/N-ABU02341.

Extraction

100 g of dried pulverized *Gliricidia sepium* was weighed and poured into a conical flask and 300 ml of ethanol was added for extraction. The sample was macerated for 12 hours and further concentrated using a rotary evaporator. After which Phytochemical (saponins, alkaloids, flavonoids, tannins, terpenoids, phenols, glycosides, and steroids) screening was conducted on the sample.

Qualitative Phytochemical test

The following methods were used to ascertain the presence of phytocompounds, alkaloid test (Dragendorff's test), tannin test (Ferric chloride test) (Sruthi *et al*., 2021), flavonoid test (Alkaline reagent test), steroid test (Gul *et al*., 2017), terpenoid test, saponin test (Emulsion test) (Ezeonu and

Ejikeme, 2016), and glycoside test (Keller-Kiliani Test) (Shah and Yadav, 2015).

Preparation of aqueous silver nitrate ($AgNO_3$)

A 1 M silver nitrate ($AgNO_3$) solution was prepared by accurately measuring 169.89 g of $AgNO_3$ using an analytical balance. The weighed sample was then dissolved in distilled water and diluted to a final volume of 1000 mL using a 1000 mL volumetric flask.

Preparation of aqueous plant extract and AgNPs synthesis

A modified version of the method described by Chhangte *et al*. (2021) was utilized for the synthesis of silver nanoparticles (AgNPs) using *Gliricidia sepium* extract. The dried plant material was ground into a fine powder using a mortar and pestle. A total of 25 g of the powdered sample was measured and mixed with 250 mL of distilled water. The mixture was stirred thoroughly and heated in a water bath at 60°C for 43 minutes. After cooling, it was filtered using Whatman No. 1 filter paper, and the filtrate was reserved for nanoparticle synthesis. For the synthesis process, 20 mL of the plant extract was combined with 180 mL of a 1 M silver nitrate ($AgNO_3$) solution in a 250 mL conical flask, maintaining a 1:9 ratio. The mixture was heated to 60 °C, stirred mechanically, and the pH was adjusted to 8 using 0.1 M Sodium hydroxide (NaOH). Reaction was allowed to proceed for 30 to 45 minutes, during which a colour change from light green to dark brown was observed, indicating the formation of green synthesized AgNPs. The solution was then left undisturbed for 24 hours to facilitate further nucleation. Subsequently, the synthesized nanoparticles were subjected to centrifugation at 2000 rpm for 20 minutes to separate the colloidal particles from the supernatant. The collected nanoparticles were oven-dried at 40°C to obtain the final product.

Characterization of Green Synthesized Nanoparticles

The synthesized silver nanoparticles (AgNPs) were characterized using Agilent Technology UV-Vis spectroscopy (Agilent Cary 300 UV-Vis-NIR), Agilent Technology Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) model (Agilent Cary 630 FTIR), XRD (Rigaku Miniflex) National steel raw materials exploration agency (NSRMEA), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray (EDX) SEM-EDX, (Phenom ProX) model at NSRMEA Malali. UV-Vis spectroscopy observed the synthesis of AgNPs, with successive dilutions of the initially prepared nanoparticle solution. For FTIR analysis, the Agilent ATR-FTIR instrument was used to collect the infrared spectrum reflecting the absorption of infrared light by the functional groups within *Gliricidia sepium* extracts and the synthesized silver nanoparticles. XRD was used for phase identification and quantification, crystallite size and strain.

SEM was employed to capture images depicting the morphology and surface characteristics of the nanoparticles, and EDX analysis identified and quantified elements on the nanoparticle surface.

In vitro assay for antitrypanosomal activity

Test organism: *Trypansoma brucei brucei*

Cryopreserved *Trypanosoma brucei brucei* stabilates were sourced from the cryobank maintained by the Vector and Parasitology Study Department at the Nigerian Institute for Trypanosomiasis Research. The stabilate was thawed at 37°C, after which its viability was assessed. Wet smears were prepared and examined under a light microscope at 400x magnification to observe trypanosome motility. The detection of actively motile parasites confirmed the viability of the trypanosomes (Bulus and Addau, 2013).

Drug incubation survival assay

The Drug Incubation Survival Test (DIST) was employed to assess the *in vitro* trypanocidal activity of the crude plant extracts and synthesized nanoparticles. For this assay, 100 µL of each reconstituted extract, nanoparticle suspension, and the reference drug were dispensed in duplicate into individual wells of a 96-well microtiter plate. Additionally, 20 µL of a blood suspension containing *Trypanosoma* spp. was introduced into each well, followed by gentle mixing to ensure uniform distribution (Okaiyeto and Okoh, 2020).

Control wells containing only 100 µL of the supplemented medium and 20 µL of the trypanosome suspension were also included to serve as a baseline for comparison. The plate was subsequently placed inside a desiccator maintaining a controlled environment of approximately 5% carbon dioxide and incubated at 37°C (Okaiyeto and Okoh, 2020).

After six hours of incubation, wet smears were prepared from each well and examined under a light microscope at 400x magnification. Trypanosome motility was observed and counted across three fields of view per sample, with a total of nine observations recorded per drug or extract concentration. Similar observations were performed for smears from the control wells. A reduction in the number of motile trypanosomes relative to the control group was used as an indicator of in vitro antitrypanosomal activity (Okaiyeto and Okoh, 2020; Bulus and Addau, 2013).

RESULTS AND DISCUSSION

Qualitative phytochemical screening of the crude extracts

Table 1: Distribution of phytoconstituents in the crude ethanol extracts of *Gliricidia sepium*

S/No.	Test	GS
1	Alkaloids	+
2	Saponins	+
3	Steroids	+

4	Tannins	+
5	Glycosides	+
6	Flavonoids	+
7	Terpenoids	+
8	Phenols	+

GS = *Gliricidia sepium*; (+) = detected; (-) = not detected

Eight classes of phytoconstituents were detected in the ethanol extract of GS namely saponins together with alkaloids, flavonoids, tannins, terpenoids, phenols, glycosides, and steroids were detected (Table 1).

Synthesis of *Gliricidia sepium* synthesized silver nanoparticles (*G.s*-AgNPs)

Upon mixing *Gliricidia sepium* extract with an aqueous silver nitrate ($AgNO_3$) solution and heating at 60°C for 30 - 45 minutes, a noticeable colour transition from light green to dark brown or reddish-brown was observed. This change in colour is indicative of the formation of silver nanoparticles and is associated with the Surface Plasmon Resonance (SPR) phenomenon, which arises from the collective oscillation of free electrons upon exposure to light (Mamman *et al*., 2023). Phytochemical compounds present in the plant extract are believed to function as reducing agents, facilitating the conversion of silver ions into nanoparticles while simultaneously stabilizing them (Al-Otibi *et al*., 2021; Kemala *et al*., 2022). The SPR effect plays a crucial role in determining the optical properties of the nanoparticles, as it depends on factors such as particle size, shape, and surrounding medium (Mamman *et al*., 2023). No additional colour changes were detected after 24 hours, signifying the completion of the nanoparticle synthesis process. This aligns with findings from previous studies, which reported a similar synthesis duration when utilizing plant extracts such as *Lippia citriodora* and *Calotropis procera* for nanoparticle production (Kero *et al*., 2017; Mamman *et al*., 2023).

UV spectrum of *G.s*-AgNPs

The UV-Vis analysis of *Gliricidia sepium*-synthesized silver nanoparticles (*G.s*-AgNPs), as shown in Figure 2, reveals an absorbance peak at 265 nm with an intensity of 1.822. This absorption occurs in the ultraviolet region, which is atypical for the surface plasmon resonance (SPR) of spherical silver nanoparticles, as SPR peaks are generally observed within the visible spectrum, typically between 380 and 450 nm. Additionally, the absence of peaks in the 335–560 nm range suggests minimal nanoparticle aggregation, a characteristic often used to confirm nanoparticle stability (Chhangte *et al*., 2021).

The observed UV-Vis absorption peak at 265 nm in the synthesized silver nanoparticles (*G.s*-AgNPs) may be attributed to several factors. One key reason is the small particle size, which can cause a blue shift in the surface plasmon resonance (SPR) band. Smaller nanoparticles exhibit quantum

size effects that alter their electronic properties, often leading to absorption in the ultraviolet region rather than the typical SPR peak observed around 400–450 nm for larger, spherical AgNPs (Nguyen *et al*., 2023). Additionally, the presence of organic compounds from the *Gliricidia sepium* extract, such as flavonoids, phenolics, or proteins, may contribute to this absorption peak. These biomolecules not only act as reducing and stabilizing agents during nanoparticle synthesis but also exhibit strong absorbance in the UV region, thereby influencing the overall spectral profile (Kumar *et al*., 2023; Chhangte *et al*., 2021). Moreover, the peak at 265 nm could result from organic capping on the nanoparticle surface, which may mask or shift the typical SPR signal. Similar UV-Vis absorption features have been reported in plant-mediated AgNPs, such as those synthesized using *Moringa oleifera*, where smaller particle sizes and organic coatings contributed to blue-shifted peaks below 300 nm (Ibrahim *et al*., 2019). In contrast, chemically synthesized AgNPs, which often lack biological capping agents and are more uniform in size and shape, typically exhibit SPR peaks around 420 nm (Choi *et al*., 2016). Therefore, the 265-nm peak observed in this study likely reflects the unique physicochemical characteristics of the biosynthesized *G.s*-AgNPs—particularly their small size, anisotropic shape, and the presence of bioorganic constituents from *G. sepium*.

ATR-FTIR spectra of synthesized *G.s*-AgNPs

Fourier-transform infrared (FTIR) spectroscopy was used to identify the functional groups responsible for capping and stabilizing the synthesized silver nanoparticles (AgNPs), with spectra recorded in the range of 4000 to 650 cm^{-1}. The FTIR spectrum presented in Figure 3 displays a distinct absorption peak at 3272.6 cm^{-1}, attributed to O-H stretching vibrations, which are characteristic of hydroxyl groups found in alcohols, phenols, or possibly water (Smith *et al*., 2020).

Additional peaks were observed at 2918.5 cm^{-1} and 2847.7 cm^{-1}, corresponding to C-H stretching vibrations, commonly associated with aliphatic groups such as CH_2 and CH_3 in alkanes or alkyl chains (Jones & Brown, 2019). The peak at 1625.1 cm^{-1} suggests C=O stretching vibrations, indicating the presence of carbonyl-containing compounds, including aldehydes, ketones, or carboxylic acids (Johnson, 2019).

Furthermore, a peak at 1543.1 cm^{-1} is linked to C=C stretching vibrations, signifying the possible presence of conjugated double bonds, often found in aromatic systems or conjugated carbonyl compounds (Garcia *et al*., 2021). The peak at 1401.5 cm^{-1} is associated with C-H bending vibrations, likely arising from aliphatic structures such as CH_2 and CH_3 groups in hydrocarbons (Smith *et al*., 2020). A peak at 1244.9 cm^{-1} suggests C-O stretching vibrations, characteristic of ether functional groups (Johnson, 2019), while the absorption at 1032.5 cm^{-1} corresponds to C-O stretching, typically found in alcohols or phenols (Jones & Brown, 2019).

Scanning electron microscope (SEM) micrographs of synthesized *G.s-AgNPs*

The scanning electron microscopy (SEM) micrograph of *Gliricidia sepium*-synthesized silver nanoparticles (*G.s*-AgNPs), as shown in Figure 4, reveals their surface morphology. The nanoparticles appear to be agglomerated, with a non-uniform distribution and a predominantly spherical shape (Kemala *et al*., 2022; Chhangte *et al*., 2021; Jabbar *et al*., 2020). Agglomeration of silver nanoparticles synthesized from plant extracts has been previously documented in various studies. Similar aggregation patterns have been observed in nanoparticles derived from *Malva parviflora* (Al-Otibi *et al*., 2021), *Crossopteryx febrifuga*, *Brilliantaisia patula*, *Senna siamea* (Kambale *et al*., 2020), *Nigella sativa* (Almatroudi *et al*., 2020), *Calotropis gigantea* (Ali and Abdallah, 2020), and *Platycodon grandiflorum* (Anbu *et al*., 2019). These findings support the tendency of biogenic silver nanoparticles to form agglomerates due to the presence of plant-derived stabilizing agents and capping molecules.

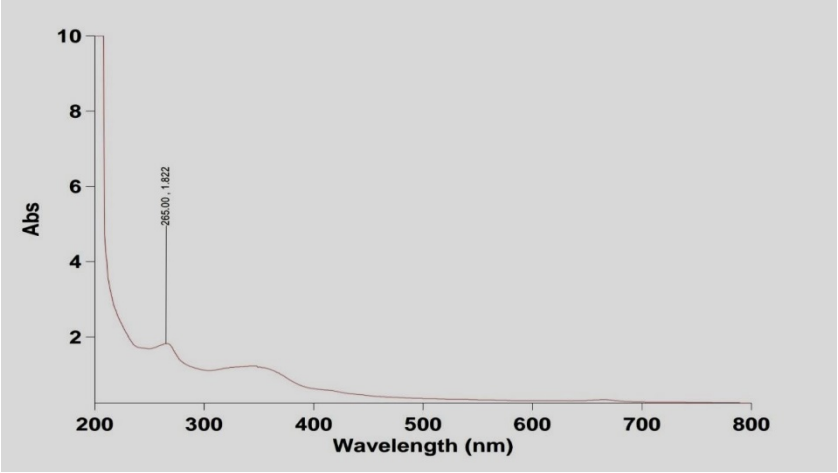

Figure 2: UV spectrum of *G.s*-AgNPs

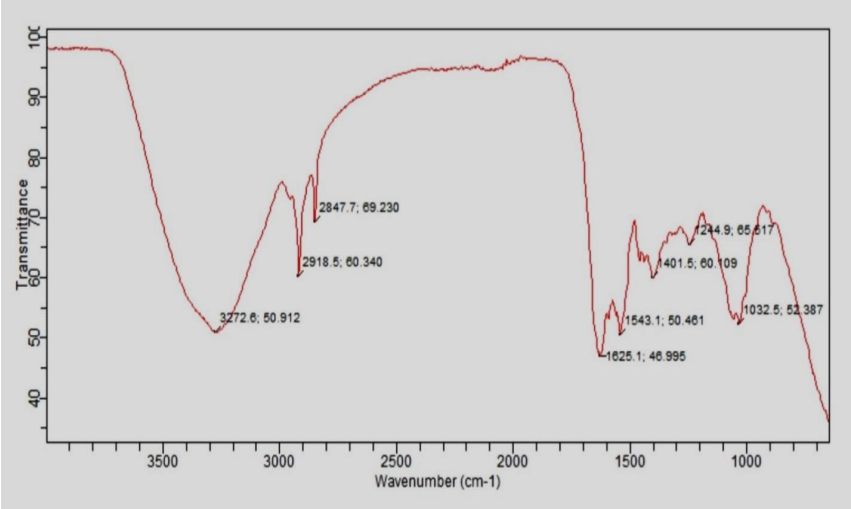

Figure 3: FTIR spectrum of *G.s*-AgNPs

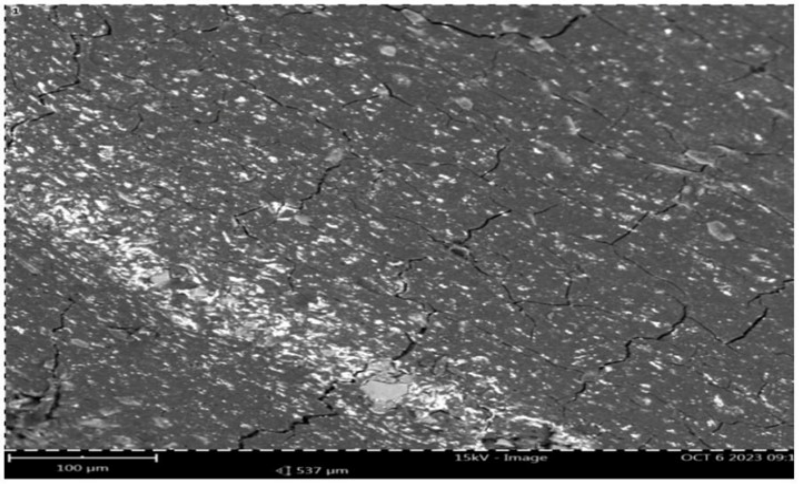

Figure 4: SEM of *G.s-AgNPs*

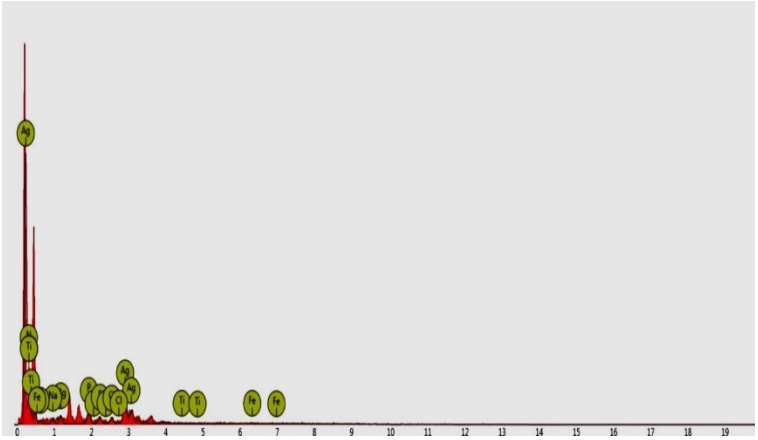

Figure 5: EDX analysis of *G.s-AgNPs*

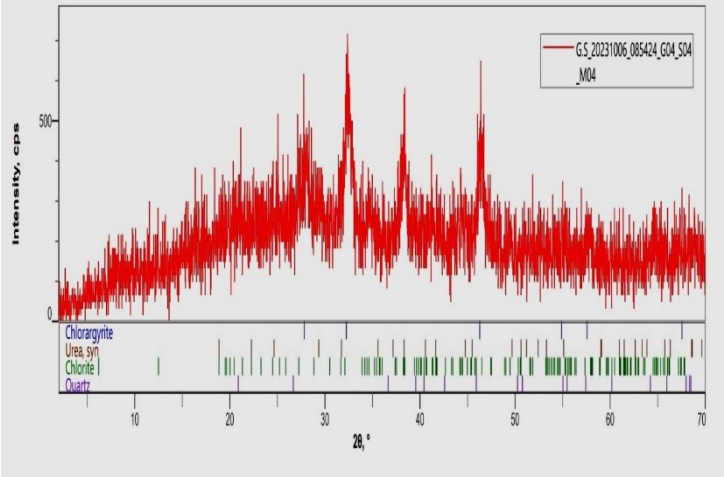

Figure 6: XRD spectrum of *G.s*-AgNPs

Energy dispersive x-ray (EDX) analysis of synthesized *G.s*-AgNPs

Energy dispersive X-ray (EDX) spectroscopy was employed to analyze the elemental composition of the synthesized *Gliricidia sepium* silver nanoparticles (*G.s*-AgNPs) as illustrated in Figure 5. The results confirmed silver (Ag) as the dominant element, constituting 46.51%, with a characteristic signal observed at 0.2 keV and within the energy range of 2.9 to 3.1 keV (Mamman *et al*., 2023; Dada *et al*., 2017). This strong metallic silver signal provides evidence of successful nanoparticle synthesis using *G. sepium*. Additionally, the EDX spectrum identified the presence of other elements, including nitrogen (N) at 28.45%, phosphorus (P) at 6.39%, sodium (Na) at 5.73%, magnesium (Mg) at 5.32%, sulfur (S) at 3.31%, chlorine (Cl) at 2.71%, iron (Fe) at 0.96%, and titanium (Ti) at 0.59%. The significant nitrogen content on the nanoparticle surface may be attributed to precursor materials or plant-derived secondary metabolites involved in the synthesis process (Mamman *et al*., 2023; Kemala *et al*., 2022)

XRD spectrum of synthesized *G.s*-AgNPs

The X-ray diffraction (XRD) spectrum of the synthesized *Gliricidia sepium* silver nanoparticles (*G.s*-AgNPs) presented in Figure 6 exhibits distinct line broadening, indicating
diffraction peaks at 2θ values of 28.04°, 32.27°, 38.38°, and 46.34°. These peaks correspond to the lattice planes (111), (200), (132), and (220), respectively. The observed diffraction patterns align with previously reported literature (Jabbar *et al*., 2020; Anbu *et al*., 2019), confirming the crystalline nature of the synthesized nanoparticles. Further analysis revealed that the identified peaks match the International Centre for Diffraction Data (ICDD) card numbers 00-001-1013 and 00-013-0003, which correspond to the structure of chlorargyrite and chlorite (AgCl, $Mg_2Al_3(Si_3Al)O_{10}(O)_8$). These structures exhibit space groups Fm-3m and C12/m1, with space group numbers 225 and 12, respectively (Alok *et al*., 2022; Ayomide *et al*., 2022). The crystallite size of the synthesized silver nanoparticles was estimated using the Debye-Scherrer equation:

$$D = \frac{K\lambda}{\beta\cos\theta}$$

Where D is the crystallite size, K is the shape factor, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the Bragg diffraction angle. Based on the most intense diffraction peak at 46.34° (corresponding to the (220) plane), the estimated crystallite size is approximately 14 nm (140 Å), with an average crystallite size of 11.7 nm (117 Å). These findings confirm that the *G.s*-AgNPs fall within the nanoscale range (Alzubaidi *et al*., 2023).

Antitrypanocidal Activity

The crude ethanol extract of *Gliricidia sepium* exhibited a significant ($p < 0.05$) concentration-dependent effect on *Trypanosoma brucei* survival. At a concentration of 10 mg/ml, the mean trypanosome count was recorded as 17.2 ± 0.98 per field. At lower concentrations of 5

mg/ml and 2.5 mg/ml, the counts increased to 18.3 ± 1.51 and 21.3 ± 1.63 per field, respectively. Further reductions in concentration (1.25, 0.625, and 0.3125 mg/ml) resulted in trypanosome counts of 21.8 ± 1.94, 22.0 ± 2.19, and 25.3 ± 1.63 per field, respectively. The control group exhibited the highest parasite count at 34.3 ± 2.34 per field. The median lethal concentration (LC_{50}) of the crude ethanol extract was determined to be 0.448 mg/ml (Table 2). Phenolic compounds such as gallic acid have shown potent inhibitory effects on *T. brucei*, with an IC_{50} of 14.2 ± 1.5 µM (Amisigo *et al*., 2019).

Table 2: Antitrypanosomal activity of *G. sepium*-synthesized silver nanoparticles (GS (syn)), crude ethanol extract of *G. sepium* (GS (EtOH)), and Diminazene aceturate (DIM) against *T. brucei.*

Concentration (mg/ml)	**GS (syn)**	**GS (EtOH)**	**DIM**
10	0.33 ± 0.52^{a}	17.2 ± 0.98^{a}	0.00 ± 0.00^{a}
5	1.50 ± 1.38^{ab}	18.3 ± 1.51^{a}	0.00 ± 0.00^{a}
2.5	2.50 ± 1.05^{b}	21.3 ± 1.63^{b}	0.00 ± 0.00^{a}
1.25	5.83 ± 1.32^{c}	21.8 ± 1.94^{b}	1.50 ± 1.05^{a}
0.625	11.2 ± 1.94^{d}	22.0 ± 2.19^{b}	4.83 ± 1.17^{b}
0.3125	15.0 ± 2.37^{e}	25.3 ± 1.63^{c}	9.50 ± 1.52^{c}
Control	34.3 ± 2.34^{f}	34.3 ± 2.34^{d}	34.3 ± 2.34^{d}
***p* value**	< 0.001	< 0.001	< 0.001
LC_{50}	0.448	8.043	0.328

Values are presented as mean ± standard deviation. Within each column, means bearing different superscript letters (e.g., a, b, c) differ significantly ($p < 0.05$) based on one-way ANOVA followed by post-hoc multiple comparison tests. Identical superscripts indicate no statistically significant difference. GS (syn) = silver nanoparticles synthesized from Gliricidia sepium; GS (EtOH) = crude ethanol extract of G. sepium; DIM = Diminazene aceturate; LC_{50} = median lethal concentration.

A similar concentration-dependent effect was observed with *G. sepium*-synthesized silver nanoparticles (*G.s*-AgNPs). At concentrations of 10 mg/ml and 5 mg/ml, trypanosome counts were significantly lower, recorded at 0.33 ± 0.52 and 1.50 ± 1.38 per field, respectively. As the concentration decreased (2.5, 1.25, 0.625, and 0.3125 mg/ml), the mean trypanosome counts increased to 2.50 ± 1.05, 5.83 ± 1.32, 11.2 ± 1.94, and 15.0 ± 2.37 per field, respectively. The LC_{50} of the synthesized silver nanoparticles was found to be 8.043 mg/ml. Recent studies have explored the trypanocidal properties of various plant extracts and compounds. For instance, research on *Vitellaria paradoxa* extracts demonstrated significant in vitro activity against *Trypanosoma brucei brucei*, with the ethyl acetate bark extract exhibiting a median inhibitory concentration (IC_{50}) of 3.25 µg/mL (Bairy *et al*., 2023).

Similarly, the reference drug Diminazene aceturate exhibited strong trypanocidal

activity. At concentrations of 10, 5, 2.5, and 1.25 mg/ml, the mean trypanosome count was 0.00 ± 0.00 per field. However, at lower concentrations of 0.625 mg/ml and 0.3125 mg/ml, trypanosome counts slightly increased to 4.83 ± 1.17 and 9.50 ± 1.52 per field, respectively. The LC_{50} value for *Diminazene aceturate* was determined to be 0.318 mg/ml. Based on LC_{50} values, Diminazene aceturate demonstrated the highest efficacy against T. brucei (LC_{50} = 0.318 mg/ml), followed by G.s-AgNPs (LC_{50} = 0.488 mg/ml), while the crude ethanol extract of *G. sepium* exhibited the least potency (LC_{50} = 8.043 mg/ml).

The trypanocidal efficacy of *Gliricidia sepium*-synthesized silver nanoparticles (G.sAgNPs) aligns with findings from other studies employing green-synthesized AgNPs. For instance, *Moringa oleifera*-mediated AgNPs have demonstrated significant antiparasitic activity, with reported LC_{50} values in the low microgram range against *T. brucei*, attributed to the synergistic effects of phytochemicals and the metallic core (Ibrahim, 2019). Compared to G.s-AgNPs (LC_{50} = 8.043 mg/ml), Moringa-based AgNPs have shown superior potency, likely due to differences in phytochemical composition, particle size, and surface chemistry. Chemically synthesized AgNPs have also exhibited strong trypanocidal activity, often with enhanced consistency due to uniform size and shape, and absence of variable plant metabolites (Choi *et al*., 2016). In this study, while *G.s*-AgNPs were not as effective as the reference drug Diminazene aceturate (LC_{50} = 0.318 mg/ml), they demonstrated superior activity compared to the crude *G. sepium* extract (LC_{50} = 0.448 mg/ml), highlighting the potential of biosynthesized nanoparticles as alternative anti-trypanosomal agents. Regarding standard treatments, Diminazene aceturate remains a commonly used antitrypanosomal agent. Studies have reported its efficacy in reducing parasitemia and ameliorating anemia in *T. brucei* infections (Jolayemi *et al*., 2020).

Conclusion

This study highlights the trypanocidal activity of *Gliricidia sepium* leaf extracts and their green-synthesized silver nanoparticles (*G.s*-AgNPs) against *Trypanosoma brucei brucei*. Both the crude ethanol extract and *G.s*-AgNPs exhibited concentration-dependent efficacy, with the nanoparticles demonstrating notably enhanced activity—likely due to improved bioavailability facilitated by nanoscale delivery. Phytochemical screening confirmed the presence of several bioactive constituents, including alkaloids, flavonoids, and saponins, which may contribute to the observed effectsNanoparticle characterization using UV-Vis spectroscopy, ATR-FTIR, SEM, and XRD validated the successful green synthesis. While the enhanced efficacy of G.s-AgNPs suggests potential for further investigation, future studies must address critical issues such as toxicity, stability, and large-scale production to evaluate their viability for therapeutic development. These findings contribute to the growing body of knowledge on plant-based trypanocidal agents and highlight *Gliricidia sepium* as a promising candidate for further drug development.

Future studies should focus on in vivo assessments, toxicity evaluations, and mechanisms of action to validate its applicability as an alternative treatment for trypanosomiasis. Recent research highlights the potential of plant-derived compounds, including those from *Gliricidia sepium*, as alternative or adjunct therapies against *Trypanosoma brucei*. Further studies are warranted to full elucidate their efficacy and mechanisms of action. The study underscores the relevance of integrating phytochemistry and nanotechnology in the search for safer, cost effective, and sustainable therapeutics for neglected tropical diseases.

Conflict of interests

The authors declare no competing interest.

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