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Acute Toxicity Studies and In Vitro Effects of *Guiera senegalensis* (Sabara) Leaf Aqueous Extract on *Trypanosoma brucei*

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ABSTRACT: Trypanosomes are a type of protozoan that is spread by the bite of an infected tsetse fly and causes nagana in animals and African trypanosomiasis (also known as sleeping sickness) in people. The purpose of this study was to identify the phytochemical components and acute lethal dose of *Guiera senegalensis* and to study the effects of its leaf aqueous extract on *Trypanosoma brucei*. Standard analysis methods were used in this study. Aside the acute toxicity (LD_{50}) test, both the phytochemical and the anti-trypanosomal potential of *Guiera senegalensis* were carried out in-vitro. While tannins, terpenoids and reducing sugars were highly present, the other phytochemicals evaluated were either moderately present or absent. The median lethal dose (LD_{50}) of the leaf aqueous extract was 500 mg/kg body weight accompanied by early clinical symptoms such as lethargy, lordosis, lack of desire for food, starry hair coat, and mortality within 24 hours. Each of these clinical manifestations was dose-dependent. *Guiera senegalensis* exerted 100% inhibition on *Trypanosoma brucei* from 0.313 mg/ml to 40 mg/ml concentration of its leaf aqueous extract. The *Guiera senegalensis* leaf aqueous extract is rich in phytochemicals and possesses anti-trypanosomal potentials.

Keywords: *Trypanosomiasis;* Lordosis; Nagana; Tannins; Terpenoids

Introduction

Trypanosomes are a type of protozoan that is spread by the bite of an infected tsetse fly and causes nagana in animals and African trypanosomiasis (also known as sleeping sickness) in people (Steverding, 2017). In Africa as a whole a protozoan species called Trypanosoma bruce causes nagana in animals and African trypanosomiasis, also known as sleeping sickness, in people (Maichomo *et al*, 2021).

Trypanosomiasis has long been considered endemic across the majority of sub-Saharan Africa, encompassing regions in roughly 37 nations with a combined population of approximately 60 million. Although the incidence has somewhat decreased in recent years, an estimated 50,000 to 70,000 persons are currently affected. For the first time in half a century, less than 10,000 cases were reported in 2009. It is thought that many cases remain undetected (Nagle *et al*., 2014). However, the disease's original range in sub-Saharan Africa has expanded to include South America, North Africa, and a sizable portion of Asia because trypanosomes adapted to mechanical transmission via blood-sucking insects (tabanids) (Diall *et al*., 2022).

Drug resistance, toxicity, and the use of costly or scarce medications are among the issues plaguing the current trypanosomiasis treatments (Barghash *et al*., 2018). Therefore, in order to tackle trypanosomiasis, it is necessary to find less toxic, more affordable, and readily available chemotherapeutic medicines. Since several ethnomedicinal plants have been shown to contain significant trypanocides, there is still great promise for using herbal formulations to treat the illness (Nekoei *et al.*, 2022).

According to reports, *G. senegalensis* has been used traditionally in several African nations to cure a wide range of illnesses. Mali, Burkina Faso, Nigeria, Guinea, Senegal, and Sudan are the countries where the majority of these studies are reported (Dirar and Devkota, 2021). According to Adam *et al.* (2024), extracts from *G. senegalensis* inhibit the development of bacterial isolates in a manner similar to that of conventional antibiotics; this finding supports the extracts' widespread usage in traditional medicine as prospective sources of antimicrobial agents. Furthermore, they stated that extracts from *G. senegalensis* include significant antioxidant components that aid in the body's scavenging of free radicals. These results highlight G. *senegalensis's* potential for creating natural antioxidant and antibacterial treatments.

Using *in vitro* techniques, this research examined the impact of the leaf of *Guiera senegalensis* aqueous extract on *Trypanosoma brucei* while also identifying the phytochemical components and acute lethal dose of the plant on the selected experimental animals.

Materials and methods

Sample collection and identification: Fresh *Guiera senegalensis'* leaves were obtained early in the morning within the University of Maiduguri Campus. The sample was determined and verified by certified botanists in the Biological Science Department, School of Sciences, University of Maiduguri Campus.

Sample processing: The leaves were properly cleaned with tap water and allowed to dry in the shade for seven days at room temperature (25 °C) in the laboratory. After being ground with a mortar and pestle, the sample was sieved to produce a 1000 g powder, which was then kept in a dried rubber container.

Sample extraction process: Four liters of distilled water were used to immerse the 1000 g of powdered *G. senegalensis* leaf, which was then shook every 6 h for 48 h. A green-coloured suspension was obtained by filtering the macerated material using muslin cloth and then again using Whatman's number one paper. This suspension was then dried at 500 °C on a hot plate, cooled in a desiccator, and mashed to produce an extract weighing 45.8 g.

Phytochemical analysis of G. senegalensis: The techniques of Trease and Evans (1989) and Sofowora (1993) were used to test for alkaloids, flavonoids, tannins, anthraquinone, steroids, carbohydrates, reducing sugar, terpenoids, glycoside, and phlabotannins. The techniques of Awe and Sodipo (2001) were used to test for the extract's saponin content, while Evans (2000) was used to test for tannins and alkaloids.

Experimental animals for LD_{50}: The animal breeding center, FUTY, Yola, provided the 15 albino rats of both sexes, weighing between 46 and 200 g, for the study. The rats were housed in an air-conditioned facility and fed commercial chicken feed as well as free access to water *ad libitum*.

Trypanosome stock: The Biochemistry Department at the University of Maiduguri Campus received the *Trypanosoma brucei* as a donation from the Nigeria Institute of Trypanosomiasis Research (NITR) in Vom, Plateau State.

Determination of the LD_{50} of G. senegalensis extract: Aliu and Nwude's (1982) modified Karber's arithmetic approach was used to determine the extract's LD_{50}. Fifteen rats in all were randomly assigned to five groups (I-V) of three rats in each group. Groups II through V received increasing intraperitoneal doses of 100, 200, 400, and 800 mg/kg body weight of the extract, respectively, whereas Group I received normal saline intraperitoneally as the control group. Over the course of a day, the rats were monitored for clinical signs and death.

The following formula was then used to determine the LD_{50}:

$$LD_{50} = LD_{100} - \frac{DD \times MD}{n}$$

where LD_{100} = the least dose that killed all the rats in a group, DD = dose difference between the groups, MD = mean death between the groups, N = number of replicate rat in the group

In vitro experiment: The leaf extract was diluted in varying quantities of phosphate buffered saline (PBS) to obtain 40 mg/ml, 20 mg/ml, 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml, 0.313 mg/ml, 0.156 mg/ml, 0.078 mg/m and 0 mg/ml in separate test tubes.

Two microliters of the parasite-containing infected blood were added to each test tube containing varying quantities of the leaf extract, and after that the tubes were placed in incubators at 37 °C to evaluate the *in vitro* anti-trypasomal activity. A glass counting chamber with a covering slip was then used to track the parasite count, which was subsequently examined under a microscope at ×40 magnification. Motile parasite counts were taken at 30 min, 1 h, 1 h 30 min, and 2 h. Using the formula below, the extract's % inhibition of the parasite at various doses was determined.

$$\%Inhibition = \frac{\text{parasite count of control} - \text{parasite count of the treated}}{parasite\ control} \ x\ 100\%$$

Statistical analysis: The mean standard deviation (S.D.) and ranges of the values were used to express each value.

Results and Discussion

The phytochemical components, median lethal dose (LD50), and in vitro activity against *Trypanosoma brucei* of the aqueous extract of Guiera senegalensis leaf were examined. The tables below display the findings.

Table 1: Phytochemical constituents of the aqueous extract of leaves of *G. senegalensis*

Component	Test	Observation	Scoring
Alkaloids	Dragendorff's	Brownish-red colour	++
Tannins	Ferric chloride	Deep red colour	+++
Flavonoids	Ferric chloride	Pale violet colour	++
Anthraquinone	Borntrigger's	None	-
Terpenoids	Lierberman-Buchard	Violet colour	+++
Steroids	Lierbrman-Buchard	Green colour	++
Saponins	Frothing	Persisted foam	++
Reducing sugar	Benedict's	Deep red	+++
Phlabotannins	Hydrogen chloride	None	-
Carbohydrate	Molish's	Red colour	++
Glycosides	Salkowski's	Blue colour	++

Keys: – = not detected, + =low concentration, ++ = moderate concentration, +++ = high concentration

In drug discovery, phytochemicals derived from medicinal plants act as a lead compound. They are then utilized in the production of synthetic or semi-synthetic drugs to guarantee patent protections (Ahmad and Ahamad, 2020).

According to the results of the phytochemical screening of the *Guiera senegalensis* leaf aqueous extract shown in Table 1 above, the leaf extract contains a significant amount of tannins, terpenoids, and reducing sugars. Additionally, during the phytochemical screening of the aqueous extract of Guiera senegalensis, anthraquinone and phlabotannins were not found, although alkaloids, flavonoids, steroids, saponins, carbohydrates, and glycosides all received moderate scores (++).

Some alkaloids are tranaquilizers, like reserpine; antimalarials, like equinine; and analgesics, like morphine (Jigam *et al.*, 2011). The presence of alkaloids in *Guiera senegalensis* leaf aqueous extract is a testament for its use in traditional medicine for treating malaria and as analgesic and tranquilizer for the body.

Many edible and inedible plants, such as tree bark, leaves, nuts, seeds, fruits, legumes, and spices, naturally contain tannins and many researches show that tannins act as antioxidant by scavenging free radicals that have the potential to damage the human liver (Makhaik *et al*., 2021). The presence of tannins in high amount in *Guiera senegalensis* leaf aqueous extract shows the potential of the extract in combating free radicals in the body.

Interest in studying natural herbal phytoconstituents has increased since they may be useful in treating diabetes. Terpenoids are among the potential substances found in plants that have been shown in numerous studies to have antidiabetic effects by improving insulin sensitivity, preventing the absorption of carbohydrates, and boosting the function of the beta cells in the pancreas. Because of their possible therapeutic benefits, terpenoids - a broad class of naturally occurring chemicals originating from plants—have drawn a lot of attention in the context of managing diabetes (Roy *et al*., 2024). Therefore, the presence of terpenoids in high concentration in *Guiera senegalensis* leaf aqueous extract as shown in Table 1 above support the application of the leaf in managing *Diabetes mellitus*.

Many glycosides, such as flavonoid glycosides, exhibit antioxidant properties by lowering oxidative stress and eliminating free radicals, which are connected to long-term illnesses like cancer and heart problems (Shen *et al*., 2022). The presence of glycosides in moderate amounts in *G. senegalensis* leaf aqueous extract supports the rich antioxidant properties of the leaf and its ability in combating free radicals in the body.

Table 2: Lethal dose (LD_{50}) of *G. senegalensis* leaf aqueous extract for albino rats

Group (n=3)	Plant Extract (mg/kg Body Weight)	Dose Difference (DD)	Dead Rate	Mean Dead (MD)	DD × MD
I	100.00		0.00	-	
				0.00	0.00
II	200.00		0.00		
		III – II = 200		0.50	100.00
III	400.00		1.00		
		IV – III = 400		2.00	800.00
IV	800.00		3.00		
Sum					900.00

$LD_{50} = LD_{100} - \frac{DD \times MD}{n} = 800 - \frac{900}{3}$ = 800 – 300

LD_{50} = 500 mg/kg bodyweight.

According to Table 2 above, the median lethal dosage (LD_{50}) of the extract from *G. senegalensis* leaf aqueous extract revealed that 800 mg/kg body weight was the dose that resulted in 100% mortality, while 400 mg/kg body weight showed 33.33% mortality. LD_{50} was determined to be 500 mg/kg body weight. There was no mortality at doses of 100 and 200 mg/kg body weight. After the albino rats received extract doses intraperitoneally, the following clinical signs were noted: lethargy, clumsiness, appetite loss, starry coat, and death within 24 h.

According to reports, substances with LD_{50} of 500–5000 mg/kg bodyweight are moderately hazardous and may be administered with some degree of safety, particularly when taken orally, where absorption may not be full because of gastrointestinal tract-inhibiting factors (Agaie *et al.*, 2000).

Tables 3 and 4 below show *in vitro* efficacy of *G. senegalensis* leaf aqueous extract on *T. bruce*i activity. There was a 100% inhibition of *T. bruce*i at different extract concentrations of 40 mg/ml, 20 mg/ml, 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml and 0.313 mg/ml. However, the extract concentrations of 0.156 mg/ml and 0.078 mg/ml showed mean percentage inhibition of 93.8 and 97.1%.

Table 3: *In vitro* efficacy of *G. senegalensis* leaf of aqueous extract on *T. bruce*i count per 10^6

Concentration of Extract (mg/ml)	Parasite Count Minutes Post Inoculation (MPS) (x 10^6)			
	30	60	90	120
PSS (Control)	2.3 ±0.09 (2.2 – 2.4)	2.2 ± 0.04 (2.2 – 2.3)	2.3 ± 0.06 (2.2 – 2.4)	2.1 ±0.02 (2.1 – 2.2)
0.078	0.1±0.02 (0.1 – 0.2)	0.1 ±0.01 (0.06 – 0.09)	0.04 ± 0.02 (0.02 – 0.07)	0.01 ± 0.01 (0.00 – 0.02)
0.156	0.1 ±0.01 (0.05 – 0.08)	0.02 ± 0.01 (0.0 – 0.03)	0.00 ± 0.00	0.00 ± 0.00
0.313	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.625	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1.250	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
10.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
40.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 4: *In vitro* efficacy of *G. Senegalensis* leaf extract on *T. bruce*i (% inhibition)

Concentration of Extract (mg/ml)	Parasite Count Minutes Post Inoculation			
	30	60	90	120
PSS (CONTROL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.078	93.8±1.23 (92.1 – 95.4)	96.3 ±0.55 (95.9 – 97.2)	98.2 ± 0.80 (97.0 – 99.0)	99.7 ± 0.39 (99.1 – 100)
0.156	97.1 ±0.61 (96.3– 97.9)	0.02 ± 0.01 (98.6 – 100)	100 ± 00	100 ± 00
0.313	100 ± 00	100 ± 00	100 ± 00	100 ± 00
0.625	100 ± 00	100 ± 00	100 ± 00	100 ± 00
1.250	100 ± 00	100 ± 00	100 ± 00	100 ± 00
2.5	100 ± 00	100 ± 00	100 ± 00	100 ± 00
5.0	100 ± 00	100 ± 00	100 ± 00	100 ± 00
10.0	100 ± 00	100 ± 00	100 ± 00	100 ± 00
20.0	100 ± 00	100 ± 00	100 ± 00	100 ± 00
40.0	100 ± 00	100 ± 00	100 ± 00	100 ± 00

Key: PSS = physiological saline solution**,** MPI = min post inoculation, Values given are mean ± standard deviation (SD) & range of 4 replicates

Trypanosoma brucei activity was 100% inhibited at various extract concentrations of 40 mg/ml, 20 ml/ml, 10 mg/ml, Smg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml, and 0.313 mg/ml, while extract concentrations of 0.156 mg/ml and 0.078 mg/ml demonstrated 93.8 and 97.1%, respectively, on the minute post inoculation of Guiera senegalensis leaf activity.

This observed anti-trypanosomal activities of aqueous extract of *Guiera senegalensis* leaf could be attributed to its phytochemical compositions. Many of these phytochemicals exhibit antioxidant properties by lowering oxidative stress and eliminating free radicals, which are connected to long-term illnesses like cancer and heart problems (Shen *et al*., 2022). The presence of glycosides, tannins and a host of other rich phytochemicals in *Guiera senegalensis* leaf aqueous extract supports the rich antioxidant properties of the leaf and its ability in combating free radicals in the body.

It is difficult to speculate the mechanism by which aqueous extract of *G. senegalensis* leaf exhibits its anti-trypanosomal activity since the bioactive components were not isolated. Nevertheless, a growing body of research indicates that a variety of natural items have antitrypanosomal properties via disrupting the parasite's redox balance, which affects the respiratory chain or the body's ability to fight off oxidative stress (Sepulveda-Boza and Cassels, 1996). This is because trypanothione reductase is extremely sensitive to changes in redox balance and natural products have structures that can produce radicals that could harm it peroxidatively. It is also known that certain substances work by attaching themselves to the parasite's kineoplast DNA.

Conclusion

The aqueous extract of *Guiera senegalensis* leaf contains moderate amounts of most of the phytochemicals evaluated and high amounts of terpenoids and tannins, both of which have been reported to exert antioxidant activities. In addition, the leaf extract exerts 100% inhibition on *Trypanosoma brucei* from 0.313 mg/ml to 40 mg/ml concentration, greatly supporting the anti-trypanosomal potential of the extract.

Conflicts of interest

There were no conflicts of interest declared by the authors.

Ethical standard of research involving animals

All institutional and national guidelines for the care and use of laboratory animals were followed.

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