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PHYTOCHEMISTRY, TOXICITY AND *IN VITRO* ANTITRYPANOSOMAL EFFICACY OF CRUDE AQUEOUS EXTRACT OF *GUIERA SENEGALENSIS* STEM BARK

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ABSTRACT

The crude aqueous extract of *Guiera senegalensis* stem bark was evaluated for its phytochemistry, acute toxicity and *in vitro* antitrypanosomal efficacy in this study. Tests for alkaloids, flavonoids, tannins, phlabotannins, saponins, steroids, cardenolides, terpenoids, cardiac glycosides, and anthraquinones were conducted. A total of 15 albino rats of both sexes were used and grouped into 5 (A to E) of 3 rats each. Groups A-D were intraperitoneally treated with graded doses of 100, 200, 400, 800mg/kg body weight of the crude aqueous extract of *G. senegalensis* stem bark. Group E was treated with Physiological Saline Solution serving as the control. All groups were observed for 24 hours for clinical signs and death to determine the median lethal dose (LD_{50}). An *in vitro* experiment was carried out with 2 drops of blood from a donor rat added to 5 ml of phosphate buffer glucose solution out of which 0.2ml was finally used at 40, 20, 10, 5, 2.5, 0.625, 0.313, 0.156 and 0.078 concentrations of the extract**.** The phytochemical screening for bioactive substances had tannins, terpenoids, alkaloids, flavonoids, saponins, anthraquinones and cardiac glycosides. Phlabotannins and cardenolides were not detected. The clinical signs observed were sluggishness, awkward posture, loss of appetite, starry hair coat and terminal death within 24 hours with LD_{50} value of 600mg/kg. The *in vitro* antitrypanosomal efficacy of the extract showed 100% inhibition of motility against *Trypanosoma brucei* at 20mg/ml. In conclusion, the crude aqueous extract of *G. senegalensis* stem bark contains phytochemical components that exhibit inhibitory trypanosomal activity.

Keywords: *Guiera senegalensis* stem bark, phytochemical components, acute toxicity, *in vitro* efficacy, *Trypanosoma brucei*

INTRODUCTION

Trypanosoma brucei, a parasitic haemoflagellate, is a blood protozoan that causes African trypanosomiasis (or sleeping sickness) in humans and Nagana in animals. There are 3 sub-species of *T. brucei* vis: *T.b. brucei, T. b. gambiense* and *T. b. rhodesiense*. It is endemic in some regions of sub-Saharan Africa, covering areas in about 37 countries containing more than 60 million people. An estimated 50,000 to 70,000 people are currently infected, however, reported cases are now below 10,000 in 2009 (WHO, 2011; 2012). The disease has spread beyond its original distribution in sub-Saharan Africa and is now present in South America, North Africa and large parts of Asia (Vanhollebeke *et al*., 2006). The existing treatments of trypanosomiasis have lots of challenges (Gutteridge, 1985). Therefore, there is a need to investigate a cheaper, more effective, easily available and less toxic chemotherapeutic agents for combating trypanosomiasis. Herbal preparations for the treatment of the disease still holds a strong potential in that some ethnomedicinal plants have been demonstrated to contain potent trypanocides (Igweh and Onabanjo, 1989; Nok, 1993; Atawodi, 2005).

Guiera senegalensis is a traditional medicinal plant used to treat various illnesses (Fiot *et al*., 2004). The branches, leaves, bark and roots of *G. senegalensis* are recommended for the treatment of stomach pain and dysenteric, syphilis, beriberi, leprosy and impotence diarrhea (Kerharo *et al*., 1948; Aniagu *et al*., 2005). The present study was therefore designed to determine the phytochemistry, acute toxicity and *in vitro* antitrypanosomal efficacy of *G. senegalensis* stem bark crude aqueous extract.

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MATERIALS AND METHODS

Plant collection and identification

The bark of *G. senegalensis* was collected within the University of Maiduguri Campus, Nigeria. The plant was authenticated by a botanist in the Department of Biological Science, Faculty of Science, University of Maiduguri, Nigeria.

Plant processing and extraction

Guiera senegalensis stem bark was thoroughly rinsed in tap water and shade dried in the Laboratory for 7 days at room temperature (25°C), ground into powder and stored in a rubber container. One thousand grams of the stem bark powder was dissolved in four liters of distilled water and then shaken every six hours for 48 hours. The solution was filtered with a muslin cloth and then refiltered using Whatman's number 1 paper and was evaporated to dryness on a water bath at 40°C.

Phytochemical analysis

Tests for alkaloids, flavonoids, tannins, phlabotannins, saponins, steroids, cardenolides, terpenoids, cardiac glycosides and anthraquinones were carried out as described by Trease and Evans (2002).

Trypanosome stock

Trypanosoma brucei was obtained from Nigeria Institute of Trypanosomiasis Research (NITR), Vom, Plateau State, Nigeria. The Trypanosome was passaged in donor rats before infection of the experimental rats, intraperitoneally with 0.1ml of saline diluted blood containing 1.5×10^6 trypanosomes. The level of parasitaemia was determined using the rapid matching method of Herbert and Lumsden (1976).

Experimental animals

Fifteen albino rats of both sexes weighing between 80 and 120g obtained from University of Maiduguri were used for the study. The rats were kept in a well-ventilated Laboratory and maintained on a commercial poultry feed (Vital® Feeds Jos, Nigeria) and drinking water *ad libitum*.

Acute toxicity study

Fifteen albino rats were grouped into 5 (A to E) of 3 rats each weighed in grams and marked for easy identification. Groups A-D were intraperitoneally treated with graded doses of 100, 200, 400, 800mg/kg body weight with a concentration of 100mg/ml of the extract based on method of Karber as modified by Aliu and Nwude (1982). Group E was treated with physiological saline solution serving as the control. The rats were observed for 24 hours for clinical signs and death. The LD_{50} of *G. senegalensis* stem bark was then calculated using the modified arithmetic method of Karber (Aliu and Nwude, 1982).

In vitro experiment

The experiment was carried out with 2 drops of blood from a donor rat added to 5 ml of phosphate buffer glucose solution out of which 0.2 ml was finally used. A serial dilution of this stock solution was done using phosphate buffer glucose to obtain descending concentrations of 40, 20, 10, 5, 2.5, 0.625, 0.313, 0.156 and 0.078 into different test tubes. Evaluation of the *in vitro* trypanosomal inhibition was performed in a test tube with 2 drops of *T. brucei* infected blood inoculated into each test tube containing the various concentrations of the extract and incubated at 37°C. Parasite count was then monitored on a glass counting chamber covered with a cover slip and observed under the light microscope at x40 magnification. The number of motile parasites was counted under the light microscope using improved Neubauer's chamber at 30 mins, l hr, l hr 30mins and 2 hrs post inoculation. The percentage inhibition of motility was calculated using this formula:

(Parasite count of control – Parasite count of treated / Parasite count of control) x 100

Statistical analysis

Inhibition of motility counts were expressed as mean ± standard deviation (SD), ranges and percentages.

RESULTS AND DISCUSSION

The phytochemistry indicating the bioactive substances present in *G. senegalensis* stem bark is shown in Table 1. The phytochemical screening for bioactive substances had indicated the presence of tannins, terpenoids, alkaloids, flavonoids, saponins, anthraquinones and cardiac glycosides. Phlabotannins and cardenolides were not detected.

Table 1. Phytochemical components of *Guiera senegalensis* stem bark crude aqueous

Component	Test	Observation	Scoring
Alkaloids	Dragendorff's	Brownish-red colour	+
Tannins	Ferric chloride	Deep red colour	+
Flavonoids	Pew's	Red colour	+
Anthraquinones	Borntrigger's	Violet colour	+
Terpenoids	Lierberman-Buchard	Violet colour	+
Saponins	Frothing	Persistence foam	+
Cardenolides	Keller – Killani	None	-
Phlabotannins	Hydrogen chloride	None	-
Carbohydrates	Molisch's	Red colour	+
Glycosides	Salkowski's	Reddish brown colour	+

Key: + = detected, - = not detected

The dose of the extract that produced 100% mortality was 800mg/kg body weight. The calculated LD_{50} was 600mg/kg body weight (Table 2). At doses of 100, 200 and 200mg/kg body weight, there was no mortality. The clinical signs observed following the intraperitoneal administration of the extract to the albino rats were sluggishness, awkward posture, loss of appetite, starry hair coat and terminal death within 24 hours.

Table 2. Median lethal dose (LD_{50}) of crude aqueous extract of *Guiera senegalensis* stem bark for albino rats

Group (n=3)	Plant extract (mg/kg)	Dose difference (DD)	Number dead	Mean dead (MD)	DD x MD
A	100		0		
		B-A=100		0	0
B	200		0		
		C-B=200		0	0
C	400		0		
		D-C=400		1.5	600
D	800		3		
E	PSS	-	-	-	-
Total					600

$LD_{50} = LD_{100} - (DD \times MD/n) = 800 - (600/3) = 600$

LD_{50} =600mg/kg body weight.

There was significant ($P< 0.05$) reduction in mean parasite count ($x\ 10^6$) with graded concentrations of 0.078, 0.313, 0.625, 1.25 and 2.50mg/ml having 3.5±0.02, 3.4±0.05, 3.3±0.04, 3.2±0.03 and 3.1±0.0 respectively compared with the normal control (PSS) with 4.0±0.1 count (Table 3).

Table 4 shows the *in vitro* efficacy of the crude aqueous extract of *Guiera senegalensis* stem bark against *T. brucei* activity. There was a 100% inhibition of motility of *T. brucei* at descending extract concentrations of 40mg/ml and 20mg/ml.

Table 3. *In vitro* efficacy of the crude aqueous extract *Guiera senegalensis* stem bark on *T. brucei*

Extract (mg/ml)	Parasite count* minutes post inoculation (MPS) ($x10^6$)			
	30 mins	60mins	90mins	120mins
PSS (contro)	4.0±0.01[a]	4.2±1.8[a]	4.5±005[a]	4.5±0.05[a]
	(4.0-4.2)	(4.2-4.5)	(4.4-4.6)	(4.4-4.6)
0.078	3.5±0.02[b]	4.0±0.01[a]	4.2±0.09[b]	4.3±0.05[b]
	(3.5-3.7)	(3.9-4.0)	(4.2-4.3)	(4.3-4.44)
0.156	3.6±0.05[a]	4.1±0.05[b]	4.0±0.06[b]	4.0±0.05[b]
	(3.5-3.6)	(3.9-4.0)	(4.0-4.1)	(4.0-4.1)
0.313	3.4±0.05[b]	3.6±0.04[b]	3.8±0.05[b]	3.8±0.04[b]
	(3.4-3.5)	(3.6-3.8)	(3.8-3.9)	(3.8-3.9)
0.625	3.3±0.04[b]	3.6±0.05[b]	3.6±0.0[b]	3.6±0.04[b]
	(3.3-3.4)	(3.6-3.7)	(3.6-3.7)	(3.6-3.8)
1.25	3.2±0.03[b]	3.6±0.04[b]	3.5±0.04[b]	3.4±0.04[b]
	(3.2-3.3)	(3.6-3.8)	(3.5-3.7)	(3.4-3.6)
2.50	3.1±0.04[b]	3.5±0.03[b]	3.2±0.02[b]	3.2±0.02[b]
	(3.1-3.3)	(3.5-3.7)	(3.2-3.4)	(3.2-3.4)
5.00	3.0±0.02[b]	3.2±0.04[b]	3.1±0.01[b]	3.2±0.01[b]
	(3.0-3.2)	(3.2-3.3)	(3.1-3.3)	(3.2-3.3)
10.0	2.8±0.01[b]	3.0±0.02[b]	3.1±0.01[b]	3.0±0.01[b]
	(2.8-3.2)	(3.0-3.2)	(3.1-3.3)	(2.8-3.0)
20.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
40.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

*Mean±SD (range) of 4 replicates; Columns mean values bearing different superscripts are statistically significant ($P< 0.05$)

Table 4. Mean ± SD (range) inhibition of motility of *T. brucei upon* exposure to *G. senegalensis* leaf crude aqueous extract

Extract (mg/ml)	Mean±SD (% inhibition) MPI			
	30 mins	60mins	90mins	120mins
PGB (Control)	00±00	00±00	00±00	00±00
0 .078	6.8 ± 3.95	6.8±3.97	5.9±3.00	3.5±0.91
	(2.4-10.0)	(2.410.0)	(3.2-8.0)	(3.4-6.0)
0.156	10.5±4.0	8.7±3.0	10.5±2.4	9.7±0.7
	(5.2-12.0)	(5.1-10.0)	(9.5-12.5)	(9.2-10.0)
0.3 13	10.2±3.01	10.2±3.0	10.5±2.40	11.5±5.6
	(6.1-13.9)	(6.1-13.9)	(8.0-13.0)	(9.0-15.0)
0.625	10.5±3.9	10.2±3.0	10.5±3.9	15.3±4.0
	(5.0-13.6)	(6.1-13.9)	(5.0-13.6)	(6.0-15.0)
1.25	14.5±4.4	12.2±5.0	15.5±4.0	16.9±2.29
	(11.5-17.5)	(10.2-15.0)	(6.0-15.0)	(13.8-19.3)
2.50	18.5±3.0	12.3±3.94	15.52±1.93	18.5±3.0
	(15.5-25.3)	(7.5-15.0)	(13.8-17.2)	(15.1-21.5)
5.0	20.5±5.0	23.5±5.4	30.5 ± 7.7	30.5±7.7
	(18.8-23.5)	(20.5-29.9)	(25.7-30.0)	(25.7-30.0)
10.0	25.7±8.5	23.6± 2.7	28.4±24.3	30.5±3.0
	(20.5-29.5)	(20.8-27.2)	(25.3-30.7)	(29.5-32.0)
20.0	100.0	100.0	100.0	100.0
40.0	100.0	100.0	100.0	100.0

PBG= phosphate buffer glucose solution, MPI= Minutes Post Inoculation
NOTE: Values are Meant ± Standard deviation (SD) and range of 4 replicates.

The phytochemical screening of the crude aqueous extract of *Guiera senegalensis* stem bark indicated presence of tannins, terpenoids, alkaloids, flavonoids, saponins, anthraquinones and cardiac glycosides. Phlabotannins and cardenolides were not detected as described by Fiot *et al*. (2004).

The median lethal dose (LD_{50}) of the aqueous extract of *G. senegalensis* stem bark was 600 mg/kg indicative of moderate toxicity (Hodge and Sterner, 1949; Bruno *et al*., 2013). The antitrypanosomal activities of the aqueous extract of *Guiera senegalensis* stem bark *in vitro* in this study could be attributed to the composition of phytochemicals. Some plant extracts have been demonstrated to contain potent trypanocidal constituents (Igweh and Onabanjo, 1989; Atawodi, 2005), and that plant trypanocidal activity should be taken within the context of the plant part and the solvent extract tested (Atawodi, 2005). It is difficult to speculate the mechanism by which these extracts exhibit their antitrypanosomal activity since the active ingredients were not isolated. However, accumulated evidence suggested that many natural products exhibit their antitrypanosomal activity by virtue of their interference with the redox balance of the parasite acting either on their respiratory chain or cellular defense against oxidative stress. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alteration in redox balance. It is also known that some agents act by binding with the kinetoplast DNA of the parasite (WHO, 2011).

In conclusion, the crude aqueous extract of *Guiera senegalensis* stem bark contains many natural products that may exhibit their antitrypanosomal activity. This result only suggests that crude aqueous extract of *G. senegalensis* stem bark has potential to provide therapeutic agents for treatment of African trypanosomiasis. Further *in vivo* studies on the crude aqueous extract of *G. senegalensis* stem bark is therefore recommended.

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