



## Research Article

### *In vitro* Anthelmintic Efficacy of Aqueous Extract of *Guiera senegalensis* J.F. Gmel (*Combretaceae*) Leaf in Maiduguri, Nigeria

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##### ABSTRACT

This study investigated the anthelmintic potential of aqueous extract of *Guiera senegalensis* leaf on earthworm (*Pheretima posthuma*) and *Strongyline* larvae cultured from sheep faeces. Graded concentrations of the extract were tested against time taken for paralysis and death of *Strongyline* larvae and earthworms. Anthelmintic activity of aqueous extract of *Guiera senegalensis* leaf based on time taken for paralysis and death of earthworm (*Pheretima posthuma*) indicated no significant difference ( $p < 0.05$ ) between the aqueous extract and albendazole control, however time taken for both paralysis and death of the earthworm decreased in a graded dose manner. Anthelmintic activity of aqueous extract of *Guiera senegalensis* leaf on mortality pattern of the earthworm (*Pheretima posthuma*) indicated that the extract concentration of 1000mg/ml compared favourably with albendazole (6.25mg), and peak anti-*Strongyline* activity was noticed at extract concentrations of 250 and 500 mg/ml as well as albendazole 6.25mg. The  $IC_{50}$  and  $IC_{99}$  values for time taken for paralysis were 19.64mg/ml and 24.79mg/ml respectively while the coefficient of determination ( $r^2$ ) was 0.3551 and the p-value was 0.4041, and the  $IC_{50}$  and  $IC_{99}$  values for the time taken for death were 21.44mg/ml and 27.47mg/ml respectively while the coefficient of determination ( $r^2$ ) was 0.3420, and p-value was 0.4152. The  $LC_{50}$  and  $LC_{99}$  values for the mortality rates were 872.73 mg/ml and 411.59mg/ml and the coefficients of determination ( $y$ ) were  $0.33x - 238$  and  $0.0551x + 76.321$  respectively. Findings of the present investigations confirm the ethno-medicinal claim of this plant.

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## INTRODUCTION

Phytomedicines are medical products of a plant or their parts such as roots, leaves, flowers, barks, seeds and fruits used to treat diseases and to improve health. They are used as complementary or alternative medicines in the prophylactic treatment and alleviation of diseases and their symptoms (Kumar and Pandey, 2013). Medicinal plants play a vital role in the treatment of various diseases as evidenced by their long history of use by all cultures (Rajeswari *et al.*, 2014). Phytomedicine has become a good alternative to synthetic anthelmintics which are mostly unaffordable to rural farmers in developing countries. *Guiera senegalensis* J.F. Gmel (*Combretaceae*) is a shrub that grows to about 3 m high and abundant in the savanna region of West Africa. *Guiera senegalensis* is one of the plants that have not been scientifically exploited fully. It is known as *kashishi* in Kanuri language, *Saabara* in Hausa language, *shafi pitu* in Marghi language and *Tadar* in Tangale language (Oshobu and Geidam, 2014). In Northern Nigeria, phytochemical screening of the aqueous leaf extract of *G. senegalensis* revealed the presence of alkaloids, tannins, flavonoids, sterols, terpenoids, and saponins. All these classes of compounds were reported to have shown important biological activities (Akuodor *et al.*, 2013). In this study, we evaluated the anthelmintic potential of aqueous extract of *Guiera senegalensis* leaf on earthworm (*Pheretima posthuma*) and *Strongyline* larvae cultured from sheep faeces

## MATERIALS AND METHODS

### Collection, Identification and Extraction of *Guiera senegalensis*

The plant *Guiera senegalensis* was identified and authenticated at the University of Maiduguri Campus by a Botanist, and a voucher specimen deposited at the Department of Biological Sciences herbarium, University of Maiduguri. The fresh leaves were air-dried under shade for 4 days, ground using pestle and mortar into fine powder that weighed 600

grams which was exhaustively extracted in 3 litres of distilled water using an Ace Soxhlet Extractor 6730 and condenser 6740 (Quick fit, England) at 60°C for 10 hours and then concentrated on an aluminium tray to remove water. A yield of 56.92g (90.51%) was obtained as dry powder and then used for the anthelmintic study.

### Collection of Faecal Sample, Culture and Isolation of *Strongyline Larvae*

Fresh faeces weighing about 20 grams each were collected from the rectum of 10 sheep at Maiduguri metropolitan abattoir into plastic test tubes, kept on ice and brought to the Department of Veterinary Parasitology and Entomology laboratory for analysis. The simple floatation technique of saturated sodium chloride solution was used to ascertain the presence of helminth eggs or ova in each faecal sample as described by Soulsby, (1982). The test tube filter paper technique of Harada and Mori (1995) was used for faecal culture and larval recovery. A 0.5g of each positive faecal sample was smeared on strips of filter paper and the lower end dipped into test tubes containing 2mls of distilled water, then covered with a clean cotton wool to stop evaporation, allowed to incubate for 10 days at room temperature, then the cotton wool and the filter paper carefully pulled out of the test tubes and the faecal solution poured into a Petri dish. The *Strongyle* larvae were identified and counted in each case using the stereoscopic microscope as described by Soulsby, (1982).

### Collection of Earthworms (*Pheritima posthuma*)

Earthworms (*Pheritima posthuma*) measuring between 2.0 and 6.5 cm in length were used for the study. These were collected from moist soils near water logged areas around the Department of Fisheries and the Faculty of Agriculture Research Farm, University of Maiduguri, Nigeria, and washed with clean water to remove all debris. *Pheretima posthuma* was used due to its anatomical and physiological resemblance with

gastrointestinal helminths (Mali and Meta, 2008).

#### ***In Vitro* Anthelmintic Activity of Extract against *Strongyline Larvae***

Descending concentrations, 500, 250, 125 and 62.5mg/ml of the aqueous extract of *Guiera senegalensis* leaf as test controls, 6.25mg/ml Albendazole (Albavet®) and distilled water as positive and normal controls were used for the *in vitro* assay in 2 replications each with 30 larvae contained in separate Petri dishes. The Petri dishes were covered with filter paper to prevent evaporation and the experiment was observed for mortality after one hour.

#### ***In Vitro* Anthelmintic Activity of Extract against *Pheretima posthuma***

Test concentrations of 700, 800, 900 and 1000mg/ml of the aqueous extract of *Guiera senegalensis* leaf, 6.25, 12.5, 25mg/ml Albendazole (Albavet®) and distilled water as positive and normal controls were used for the *in vitro* assay in 2 replications each with 5 earthworms contained in separate Petri dishes, observed for paralysis and death after one hour. Paralysis was said to occur when the worm was shaken vigorously and neither moved nor responded to stimuli and death was as a result of colour change of the earthworm to pale white.

#### **Data Analysis**

Data obtained were expressed as mean time of paralysis and death, and mortality rates. Probit analysis was used to calculate the LC<sub>50</sub> and LC<sub>99</sub> values for the mortality rates and IC<sub>50</sub> and IC<sub>99</sub> values for time taken for paralysis and death of earthworms.

## **RESULTS**

Anthelmintic activity of aqueous extract of *Guiera senegalensis* leaf based on time taken for paralysis and death of earthworm (*Pheretima posthuma*) is shown in Table 1. No significant difference ( $p > 0.05$ ) was observed between the aqueous extract and albendazole control. The time taken for both paralysis and death of the earthworm's decreased in a graded dose manner. Table 2 shows the anthelmintic activity of aqueous extract of *Guiera senegalensis* leaf on mortality pattern of the earthworm (*Pheretima posthuma*). The extract concentration of 1000mg/ml compared favourably with albendazole (6.25mg). Table 3 shows the anthelmintic activity of aqueous extract of *Guiera senegalensis* leaf on *Strongyline* larvae cultured from sheep faeces and their control. Peak anthelmintic activity was noticed at extract concentrations of 250 and 500 mg/ml as well as albendazole (6.25mg). Figure 1 and Figure 2 shows the linear regression line of relationship between graded extract concentrations and mortality rate of *Pheretima posthuma* and *Strongyline* larvae. The LC<sub>50</sub> and LC<sub>99</sub> values for the mortality rates were 872.73 mg/ml and 411.59mg/ml and the coefficients of determination ( $y$ ) as  $0.33x - 238$  and  $0.0551x + 76.321$  respectively. Figure 3 shows the IC<sub>50</sub> and IC<sub>99</sub> values for time taken for paralysis as 19.64mg/ml and 24.79mg/ml respectively while the coefficient of determination ( $r^2$ ) was 0.3551. The p-value was 0.4041.

Figure 4 shows the IC<sub>50</sub> and IC<sub>99</sub> values for the time taken for death as 21.44mg/ml and 27.47mg/ml respectively while the coefficient of determination ( $r^2$ ) was 0.3420. The p-value was 0.4152.

**Table 1:** Anthelmintic activity of aqueous extract of *Guiera senegalensis* leaf on time taken for paralysis and death of the earthworm (*Pheretima posthuma*)

Concentrations mg/ml	Time taken (minutes)	
	Mean $\pm$ S.D	
	Paralysis	Death
<b>Leaf Aqueous Extract</b>		
700	0 <sup>b</sup>	0 <sup>d</sup>
800	41.0 $\pm$ 0.00 <sup>c</sup>	44.0 $\pm$ 2.12 <sup>b</sup>
900	31.57 $\pm$ 1.72 <sup>c</sup>	32.29 $\pm$ 1.98 <sup>c</sup>
100	30.56 $\pm$ 1.13 <sup>c</sup>	32.50 $\pm$ 1.31 <sup>a</sup>
<b>Albendazole</b>		
25	28 $\pm$ 1	35.2 $\pm$ 1.48
12.5	36.8 $\pm$ 1.64	41 $\pm$ 1.58
6.25	37.4 $\pm$ 1.14 <sup>c</sup>	50.2 $\pm$ 1.30 <sup>b</sup>
<b>Distilled water</b>	0 $\pm$ 0	0 $\pm$ 0

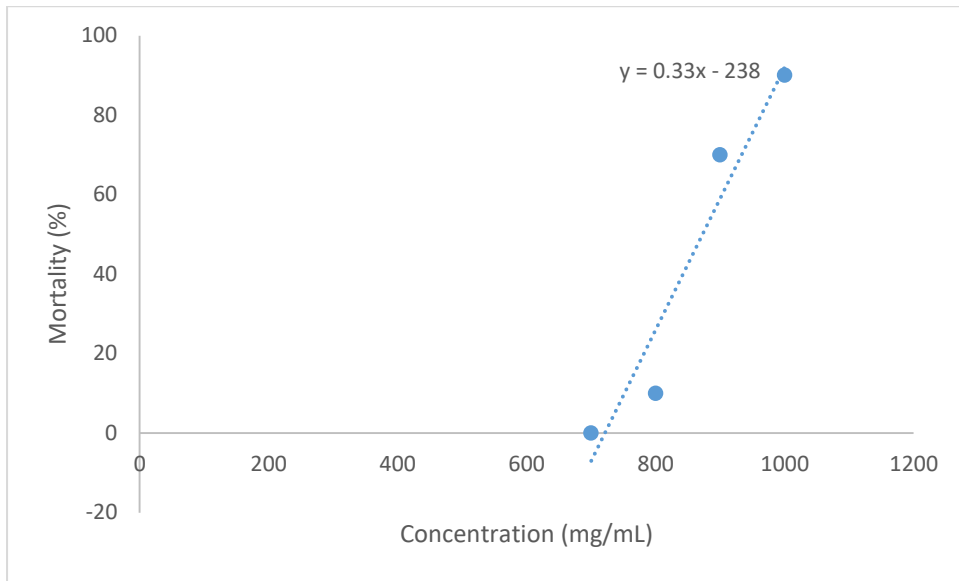
Columns with different superscripts differ significantly ( $p < 0.05$ ) with respect to albendazole at the least dose.

**Table 2:** Mortality Rates of *Pheretima posthuma* based on Graded Extract Concentrations after One Hour

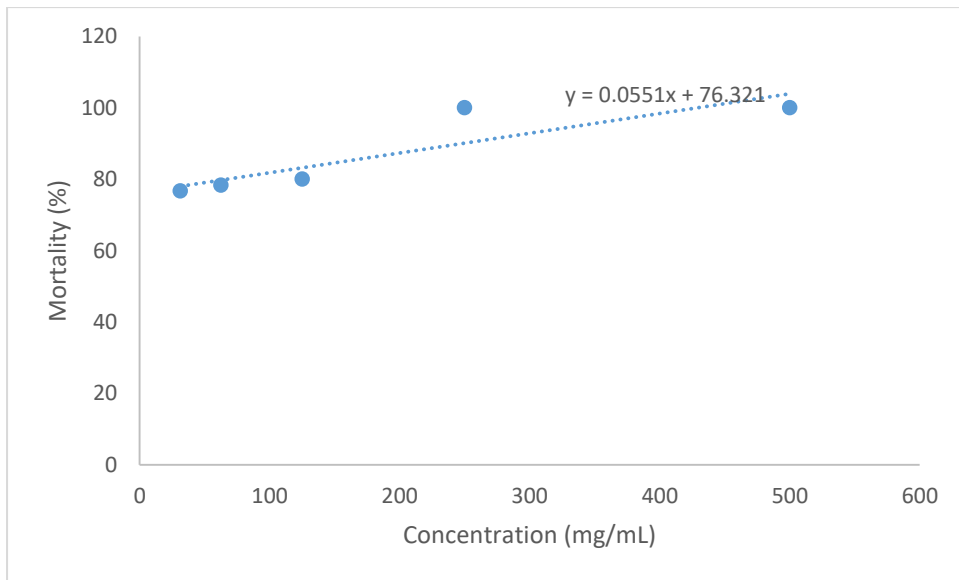
Concentrations (mg/ml)	No. (%) Dead (N=10)
700	0(0)
800	1(10)
900	7(70)
1000	9(90)
Albendazole	10(100)
Distilled Water	0(0)

**Table 3:** Mortality Rates of *Strongyline* Larvae based on Graded Extract Concentrations after One Hour

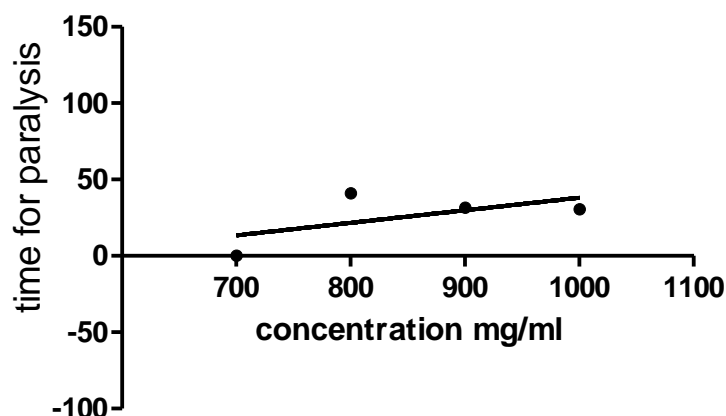
Concentrations (mg/ml)	No. (%) Dead (N=60)
500	60(100)
250	60(100)
125	48(80)
62.5	47(78.3)
31.25	46(76.7)
Albendazole (6.25)	60(100)
Distilled Water	0(0)



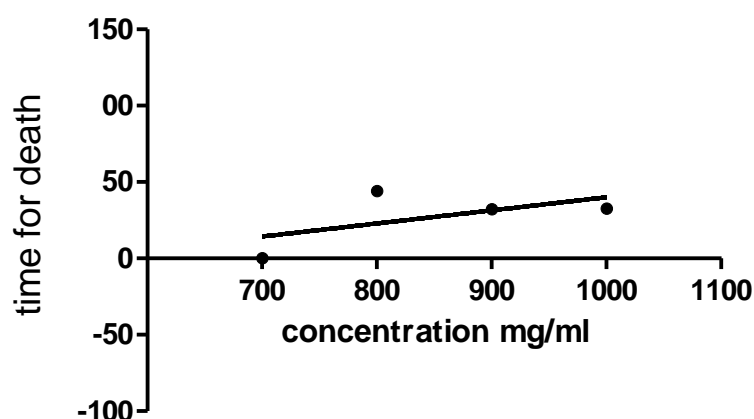
**Figure 1:** Linear regression showing the relationship between Graded Extract Concentrations and Mortality Rate of *Pheretima posthuma*



**Figure 2:** Linear regression showing the relationship between Graded Extract Concentrations and Mortality Rate of *Strongyline Larvae*



**Figure 3:** Regression equation of time of paralysis of earthworms against concentration



**Figure 4:** Regression equation of time of death of earthworms against concentration.

## DISCUSSION

Africa is one of the richest in terms of phyto-diversities in the world. The consumption of plant materials is believed to contribute immensely to the improvement of the health of man and animals, and it has been estimated that 8% of the population of Africa depend on medicinal plants to satisfy their healthcare requirements (Okaiyeto and Oguntibeju, 2021). Phytomedicine has become a good alternative to synthetic anthelmintics and a variety of *in vivo* and *in vitro* methods has been used to evaluate and validate anthelmintic properties of plant remedies (Adedapo, *et al.*, 2007).

This study investigated the anthelmintic potential of aqueous extract of *Guiera senegalensis* leaf on earthworm (*Pheretima posthuma*) and *Strongyline* larvae cultured from sheep faeces. Anthelmintic activity of aqueous extract of *Guiera senegalensis* leaf on

mortality pattern of the earthworm (*Pheretima posthuma*) indicated that the extract concentration of 1000mg/ml compared favourably with albendazole (6.25mg), and peak anti-*Strongyline* activity was noticed at extract concentrations of 250 and 500 mg/ml as well as albendazole 6.25mg. Similar observations with the use of medicinal plants have been made by Partap *et al.*, (2012). The other test concentrations of the extract showed marked degree of anthelmintic activity. Graded concentrations of the extract were tested against time taken for paralysis and death of *Strongyline* larvae and earthworms. The anthelmintic activity could be tied to the presence of phytochemicals present in the plant. Phytochemicals have been demonstrated to possess anthelmintic activities (Ekeanyawu and Etienajirhevwe, 2012). *Guiera senegalensis* leaf extracts has been shown to contain alkaloids, flavonoids, terpenoids,

polyphenols, tannins, carbohydrates, proteins, steroids, anthraquinones and saponins (Sule *et al.*, 2002; Sombié *et al.*, 2011 Shafei *et al.*, 2016). Alkaloids, flavonoids, saponins and tannins have been demonstrated to possess anthelmintic activities (Ekeanyawu and Etienajirhevwe, 2012). Some are shown to produce anthelmintic activities due to interference with energy generation by uncoupling parasite specific fumarate reductase- mediated oxidative phosphorylation reactions. Some also bind to free proteins in the gastrointestinal tract of the host or to the glycoproteins on the cuticle of the parasite which caused death (Raza *et al.*, 2016). Albendazole is a proprietary antihelminthic that belongs to the benzimidazole group, and has been shown to affect worms by destroying the cytoskeletal structure of the worm thereby causing paralysis. They act by uncoupling mitochondrial function associated with electronic transport to enable ATP generation (Adedapo, *et al.*, 2007). Findings of the present investigations confirm the ethno-medicinal claim of this plant.

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## CONCLUSION

The anthelmintic potential of aqueous extract of *Guiera senegalensis* leaf on earthworm (*Pheretima posthuma*) and *Strongyline* larvae cultured from sheep faeces was investigated. The findings from the study suggest that the extract possess anthelmintic activity with death of the earthworm and *Strongyline* larvae

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## Authors Contribution

Abdullahi A. Biu, Hauwa S. Bello, Maimuna A. Abdulkadir, and ThankGod E. Onyiche conceived, designed, and coordinated the study, while Jummai Musa conducted the experiments assisted by the appropriate laboratory staff.

## Conflict of Interest

There is no conflict of interest as regards to this manuscript

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