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Anthelmintic efficacy of aqueous extract of *Guiera senegalensis* on earthworm and *strongyle* larvae from goat faeces in Maiduguri

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Abstract

Guiera senegalensis (Combretaceae), is a small shrub abundant in semi-desert areas of the Sudano-Sahelian zone and used widely for the treatment of many complications such as gastrointestinal disorders, microbial, respiratory and parasitic infections. There is renewed interest in the use of medicinal plants to control gastrointestinal helminths of medical and veterinary importance. This study was aimed at investigating the anthelmintic potential of aqueous leaf extract of *Guiera senegalensis* (Sabara) on earthworm and strongyline larvae cultured from goat faeces. Four different concentrations (700, 800, 900 and 1000 mg/mL) of the extracts were tested and results were expressed in terms of time for paralysis and death of the earthworms and mortality of L_3 larvae in 60 minutes. Albendazole was used as a reference standard using three different concentrations (25, 12.5 and 6.25 mg/mL). A Dose-dependent activity was observed in the plant extracts on both the earthworms and L_3 larvae. Peak activity was 1000 mg/ml for the earthworm and 250 mg/ml for the L_3 larvae. Half-maximal inhibitory concentration (IC_{50}) for paralysis and death of the earthworms was 14.01 mg/ml and 14.64 mg/ml respectively. The mortality index and larval mortality percentage of the L_3 larvae for the 250 mg/ml of the aqueous extract was comparable with that of Albendazole. Findings from this study confirm the folkloric claim of the anthelmintic potential of this plant.

Keywords: Anthelmintic, Death, Leaf extract, Mortality index, Paralysis, Peak activity

Introduction

Parasitic gastroenteritis caused by nematodes is an important economic disease in goats in Nigeria. It is one of the major constraints in livestock production. The humid climate is favourable for the development of the free-living stages of Trichostrongyle nematode (Belina *et al*., 2017). The mortality rate is particularly high in young ruminants, but adult animals also suffer considerably from chronic helminthosis (Roeber *et al.*, 2013). Intensification of animal production systems has led to an increasing demand for effective anthelmintic drugs to control helminth diseases. Regular dosing of susceptible animals has been shown to result in the development of resistance to nematodes (Coles *et al.*, 2006; Sutherland &

Leathwick, 2011). This is a subject, in recent years has attracted considerable attention from scientists of diverse backgrounds, but as it is with most evolving conditions, it gained prominence and notoriety only when either health or economic development is threatened. Given the long lead time required in producing a vaccine against parasites and the complexity of host-parasite association, anthelmintics will continue to play a central role in nematode control programs of livestock for the foreseeable future. Anthelmintics are drugs intended to act against parasites that inhabit the gastrointestinal tract, its associated structures and such organs as the liver, lungs and the blood circulatory system. Chemicals that can be used to remove the endoparasites without side effects on the host animals or man are difficult to discover and develop (Prichard, 1990; Ademola *et al*., 2007). Various compounds have been used since the beginning of this century, in an attempt to control helminth parasites.

Herbs are defined as crude drugs of vegetable origin utilized for the treatment of disease states, often of a chronic nature, or to attain or maintain a condition of improved health (Chan, 2003; Che *et al*., 2017). Pharmaceutical preparations made by extracting herbs with various solvents to yield tinctures, fluid extracts, or the like are known as phytomedicinals. About 92 varieties of plants and other materials have been used in Nigeria to treat and prevent livestock health problems (Ademola, 2016). It has also been demonstrated that preparations made from methanolic or ethanolic leaf galls possess antiviral properties (Aniagu *et al*., 2005). Other people reduce the galls to powder with the pith from *Combretum aculeatum* stems and salt. This powder is diluted in water immediately before use and is prescribed for painful stomach cramps with mucous stools and vomiting (Kerharo & Adam, 1974; Zakawa *et al*., 2018). A tea made from leaves of *G. senegalensis* is prescribed by oral route to treat eczema (one litre per day), and is also used against attacks of fever, and to cure chest conditions and colds (Malgras, 1992; Zakawa *et al*., 2018). Fresh mashed, chewed or cut leaves of *G. senegalensis* when placed on a wound bleeding (Berhaut, 1967; Somboro *et al*., 2011). The leaves are also used as a poultice on tumours and against the Guinea worm (Berhaut, 1967; Somboro *et al*., 2011). Besides these uses in traditional medicine, some extracts of this plant have been found to possess pharmacological properties: antimicrobial (Le Grand, 1989; Bosisio *et al*., 1997; Sanogo *et al.,* 1998), antifungal (Silva & Elsa, 2003), antioxidant (Bucar *et al.,* 1998; Bouchet *et al*., 1998) and trypanocidal effects (Aderbauer *et al*., 2008). The aim of this study is to determine the anthelmintic activity of aqueous leaf extract of *Guiera senegalensis* on earthworm (*Pheretima posthuma*) and strongyle larvae cultured from goat faeces in Maiduguri, Nigeria.

Materials and Methods

Plant collection and identification

Leaves of *Guiera senegalensis* were obtained from the botanical garden of the University of Maiduguri. The leaves were identified by a botanist from the Department of Biological Science and shade dried. Thereafter, the dried leaves were pulverized into a fine powder using pestle and mortar.

Plant processing and extraction

The Plant extract yielded 56.92 g from six hundred grams (600g) of fine powder of *Guiera senegalensis* with three (3) litres of distilled as described by WHO (1992). The plant extract was stored at 4^0C until use.

Experimental design

Faecal samples of sheep were collected from Maiduguri abattoir in labelled polythene bags and transported to the Veterinary Parasitology and Entomology laboratory of the Faculty of Veterinary Medicine, University of Maiduguri within one hour of sample collection for analysis

Floatation technique was used to verify the presence of helminth eggs in the samples as described in Soulsby (1986). Faecal culture and larval recovery were done using the test tube paper technique as described by Harada & Moris (1955) while identification and counting of the infective larval stage were done using the protocol of Soulsby (1982). The earthworms about 2.0 to 6.5 cm in length used for the study were collected from the water-logged areas near the Department of Fisheries and the Agriculture Research Farm of the University of Maiduguri where moist soils are available. After collection from the moist soil, they were washed with water to remove all debris. *Pheritima pasthuma* was used due to its anatomical and physiological resemblance with parasitic gastrointestinal nematodes of both humans and animals (Nirmal *et al*., 2007).

The anthelmintic activity was performed using standard protocol as described by Ajaiyeoba *et al*. (2001). About 0.5ml of a known quantity of third-stage larvae (L3) was suspended in distilled water were exposed to 1ml of varying concentrations of *Guiera senegalensis* aqueous leaf extract (250, 125,

62.5 and 31.25mg/ml) using a clean Petri dish in duplicate. Distilled water and Albendazole (Albavet®) at different concentrations (6.25, 12.5 and 25mg/ml) were used as control and standard drug respectively in duplicate. The Petri dishes were covered with filter paper to prevent evaporation and the experiment was observed for one (1) hour period. The number of dead and live larvae was recorded. The number of dead larvae was ascertained by subtracting the number alive from the total number of larvae in each Petri dish before the commencement of the assay. Percentage inhibition of larval development was calculated using the formula described by Coles *et al*. (1992) & Bizimenyera *et al*. (2006) with slight changes:

Larval mortality percentage (%) = $100(1-X_1/X_2)$

Where X_1 is the initial number of larvae in test extracts pre-treatment, and X_2 is the number of larvae that were obtained post-treatment.

Mortality index was also calculated for each concentration of the extract as well as the standard drug (Albendazole) using the formula

Mortality index = Total number of larvae after treatment/Total number of larvae before treatment

Assessment of anthelmintic efficacy of the extract on Earthworm (*Pheritima posthuma*).

The anthelmintic activity was performed using a standard protocol as described by Ajaiyeoba *et al*. (2001). Approximately, 5-7 earthworms were placed in Petri dishes and exposed to four different concentrations (700, 800, 900 and 1000mg/ml) of aqueous extract of *Guiera senegalensis* leaf extract in duplicates. Distilled water and Albendazole (Albavet®) (6.25, 12.5 and 25mg/ml) served as control and standard drug in duplicates. The earthworms were observed for paralysis and death over a one (1) hour period. Death was ascertained by non-responsiveness to stimuli and the pale white colour change of the earthworm. The mean time for paralysis and death was noted. The number of dead and alive was also recorded.

Statistical analysis

Analysis of variance (ANOVA) was used to determine the extent of variation among groups and $p \leq 0.05$ was considered significant (Mead & Curnow, 1983). The results were expressed as mean ± standard deviation.

Results

Table 1 shows the anthelmintic activity of the aqueous leaf extract of *Guiera senegalensis* on L_3 larvae cultured from goat faeces and their control. A Significant difference ($p<0.05$) was observed between the different treatment groups of the extracts compared with the standard drug with respect to the mean number of L_3 larvae cultures from goat faeces. in other words, the mean number of live L_3 larvae decreased as the concentration of the extract increased. Furthermore, the mortality index and percentage inhibition increased as the concentration of the extract increased. Albendazole (standard drug) and 250mg of aqueous leaf extract of *Guiera senegalensis* both had a mortality index and larval mortality percentage of 1 and 100% respectively.

Anthelmintic activity of aqueous leaves extract of *Guiera senegalensis* based on time for paralysis and death of the Earthworm and their control is presented in Table 2. A significant difference ($p<0.05$)

Table 1: Anthelmintic activity of aqueous leaves extract of *Guiera senegalensis* cultured from goats faeces

Concentration of *Guiera senegalensis* extract	Mean ± S.D		Mortality index	Larval Mortality index
	Alive	Dead		
31.25 mg/ml	9±5.66^{c}	19±2.83^{d}	0.69	62
62.5 mg/ml	6±2.83^{b}	19±4.24^{a}	0.88	36
125 mg/ml	2±1.41^{a}	17.5±4.95^{c}	0.88	21
250 mg/ml	0±0^{d}	34±7.07^{b}	1	100
Albendazole (Standard drug)				
6.25 mg/ml	0±0^{d}	35±15.55^{b}	1	100
12.5 mg/ml	0±0	32.5±17.67	1	100
25 mg/ml	0±0	22.5±4.95	1	100
Distilled water (control)	29±15.56	0±0	0	0

a,b,c,d rows with different superscripts differ significantly ($p<0.05$) with respect to standard drug (control) at the least dose

Table 2: Anthelmintic activity of aqueous leaves extract of *Guiera senegalensis* on time taken for paralysis and death of the earthworm

Concentrations of aqueous leaves extract of *Guiera senegalensis*	Time taken (minutes) Mean±S.D	
	Paralysis	Death
700 mg/ml	0^{b}	0^{a}
800 mg/ml	28.5 ± 0.70^{c}	31.5 ± 2.12^{a}
900 mg/ml	26.8 ± 0.83^{c}	29 ± 1.22^{c}
1000 mg/ml	21.22 ± 1.20^{b}	24.42 ± 1.27^{a}
Albendazole (standard drug)		
25 mg/ml	28±1	35.2±1.48
12.5 mg/ml	36.8±1.64	41±1.58
6.25 mg/ml	37.4 ± 1.14^{c}	50.2 ± 1.30^{b}
Distilled water (control)	0±0	0±0

a,b,c,d rows with different superscript differs significantly ($p<0.05$) with respect to standard drug (control) at the least dose

Table 3: Anthelmintic activity of aqueous leaves extract of *Guiera senegalensi*n mortality pattern of the earthworms

Concentration of the extract	Mean±S.D	
	Alive	Dead
700 mg/ml	5.0±0	0±0
800 mg/ml	4.0±0	1.0±0
900 mg/ml	2.5±0.70	2.5±0.70
1000 mg/ml	0.5±0.70	4.5±0.70

was observed at lower doses in terms of time taken for the death of earthworms exposed to the extract at different concentrations. The time taken for both paralysis and death of the earthworms decreased in a graded dose manner. Table 3 shows the antihelmintic activity of the aqueous leaf extract of *Guiera senegalensis* on the mortality pattern of the earthworms. The mean number of earthworms alive after exposure to the extract for one hour decreased in a graded dose manner, while the mean number of dead earthworms increased in a graded dose manner.

Discussion

Plants with promising bioactive compounds should be scientifically validated through systematic experiments. In this study, the aqueous leaf extract of *Guiera senegalensis* was screened for its *in vitro* anthelmintic activity. The extract on earthworms had maximum anthelmintic activity at the highest concentration of 1000 mg/ml where it caused paralysis and death. , The extract also had a mortality index of 1 and larval mortality percentage of 100% against the 3rd stage larvae at the highest concentration of 250 mg/ml comparable with the standard drug, albendazole. The anthelmintic activity is linked to the presence of phytochemicals present in the plant, particularly, tannins and saponins which are known to possess anthelmintic activity. The mean reduction in the number of earthworms after exposure to the extract for one hour decreased in a graded dose manner, buttressing the general assumption of a graded dose-response to all extracts. The branches, leaves, bark and roots of *Guiera senegalensis* have been recommended for the treatment of stomach pain and dysenteric diarrhoea (Alshafei *et al*., 2016), syphilis, beriberi, leprosy and impotence (Zakawa *et al*., 2018). It is also used in veterinary medicine among the Tukolor people in diets designed to increase body weight, reproductive capacity and milk secretion in animals (Kerharo & Adam, 1974; Somboro *et al*., 2011). Similar activity has been reported by other scientists with the use of plant extract (Maphosa *et al*., 2010).

In conclusion, the results of this study have shown a promising anthelmintic activity of *Guiera Senegalensis* suggesting the possible use of aqueous leaves extract of *Guiera Senegalensis* extracts in intestinal nematode control. These findings support the folkloric use of the plant by locals in the treatment of helminth infections in both humans and animals.

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Conflict of Interest
The authors declare that there is no conflict of interest.

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