Anthelmintic effects of aqueous extract *Balanites aegyptiaca* stem bark on strongyle larvae and the earthworm *Pheretima posthuma*

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Abstract

The development of anthelmintic resistance and the high cost of conventional anthelmintics have led to the evaluation of medicinal plants as an alternative source of anthelmintics. In the current study, *in vitro* experiments were conducted to determine the possible anthelmintic effects of *Balanites aegyptiaca* stem bark aqueous extract on Strongyle larvae and the earthworm *Pheretima posthuma*. The *in vitro* studies revealed that the extract at graded concentrations of 300 mg/ml, 400 mg/ml, 500 mg/ml and 600 mg/ml exhibited larvicidal activity (p<0.05) against earthworms with 600 mg/ml having the highest activity causing paralysis and death. In a similar vein, a graded concentration response was observed against Strongyle larvae. The highest effective concentration on larvae was 250 mg/ml which had a larval mortality rate of 100% and a mortality index of one (1) comparable with that of albendazole. The IC⁵₀ and IC⁹₉ values for the time of paralysis were 442.7mg/ml and 767.80mg/ml respectively. The anthelmintic activity exhibited by the extract could be linked to the presence of phytochemicals present in the plant. These findings support the folkloric use of this plant in the control and management of gastrointestinal nematodes in humans and animals.

Keywords: Anthelmintic Effects, Aqueous Extract, *Balanites aegyptiaca*, *Pheretima posthuma*, Strongyle Larvae

Introduction

One of the most common infections that affect livestock is gastro-intestinal parasitism and the clinical signs and sequelae are dependent on the parasite fauna present and the intensity of infection. In sheep, these can range from sub-clinical weight loss to lethal pathologies such as anaemia, diarrhoea and severe protein loss (Pugh & Baird, 2012). In the last six decades, anthelmintics have become an important strategy to control nematode infections in livestock for increased production (Kaplan, 2004). However, the development of resistance to synthetic chemical anthelmintics has led to the need to search for alternative biomolecules and hence medicinal plants are been explored for potential lead compounds (Lateef *et al.*, 2013), that could be developed further for new pharmaceutical products with potent anthelmintic activity (Diehl *et al.*, 2004).
Sheep represent an important source of income in many countries including Nigeria and the effects of parasitism on their production have been well documented (Charlier et al., 2014). Anthelmintic resistance and climate change are likely to alter the geographical distribution of parasites and their impact on production animals, thus increasing the need for a clear understanding of the cost of parasitism to develop sustainable control strategies (FAOSTAT, 2013).

Desert date commonly referred to as *Balanites aegyptiaca*, (L.) Del. belongs to the family Zygophyllaceae. The plant is commonly distributed in the Sudano-Sahelian region of Africa, the Middle East and South Asia and the fruit is a rich source of nutrients and has a wide range of nutraceutical applications (Elfeel, 2010; Chothani & Vaghasiya, 2011). The anthelmintic activity of methanolic extract of *B. aegyptiaca* fruits has been evaluated against different parasites including *Toxocara vitulorum*, *Paramphistomum microbothrium*, *Trichinella spiralis* (Shalaby et al., 2010; Shalaby et al., 2012; Shalaby et al., 2016), *Schistosoma japonicum* and *Fasciola gigantica* (Koko et al., 2000). Several methods have been developed for evaluating *in vitro* nematocidal activity of plant extracts. These assays are based on the assumption that a nematocidal activity observed *in vitro* is indicative of potential *in vivo* activity. Therefore, this current study was carried out to assess the *in vitro* anthelmintic effects of *B. aegyptiaca* stem bark aqueous extract on the earthworm *Pheretima posthuma* and *Strongyle* larvae compared with Albendazole (Albavet®).

**Materials and Methods**

**Plant collection and identification**

A fresh sample of *Balanites aegyptiaca* stem bark were collected in the evening from University of Maiduguri campus, Borno State, Nigeria. It was identified and authenticated at the herbarium of the department of Biological Sciences, University of Maiduguri. The fresh stem bark was air dried under shade for 4 days and they were ground into fine powder using a pestle and mortar.

*Balanites aegyptiaca* stem bark processing and extraction

Six hundred grams (600g) of the pulverized stem bark was exhaustively extracted in three litres of distilled water using an Ace Soxhlet Extractor 6730 and Condenser 6740 (Quick Fit, England) at 60°C for 10 hrs. The extract was concentrated on an aluminium tray, placed into an oven and maintained overnight at 60°C as a drying process to remove water. A yield of 56.92 g was obtained as dry powder using the formula as described by Sasonko et al. (2011)

\[
\text{Percentage extraction} = \frac{\text{Amount of dried plant used (g)}}{\text{Amount of extract yield (g) X 100}}
\]

The extract was stored as a stock solution in a refrigerator until used.

**Evaluation of anthelmintic activity of the aqueous extract on *Pheretima posthuma***

*Pheretima posthuma* were collected from moist compost soils within the premises of the Departments of Fisheries and the Animal Farm, University of Maiduguri, Nigeria. They were rinsed with distilled water to remove all debris. The anthelmintic activity was performed using a standard protocol as described by Ajaiyeoba et al. (2001). *Balanites aegyptiaca* stem bark aqueous extract concentrations of 300, 400, 500 and 600 mg/ml were used for the *in vitro* assay. For each concentration, two petri dishes were prepared to make a total of eight petri dishes. A further duplicate was also prepared with distilled water as normal control. Albendazole (Albavet®) at concentrations 6.25 mg/ml, 12.5 mg/ml and 25 mg/ml a standard commercial anthelmintic drug were also prepared in duplicates to serve as positive controls. Each of these test Petri dishes was exposed to 5 earthworms and were observed for time to paralyze noticed by movement failure unless plagued by touch or immersed in warm water (50°C) and time to death noticed by a complete absence of movement upon stimulation or immersion in warm water coupled with white secretion and fading away of their body colour. Either the time to paralyze or time to death indicated anthelmintic activity (Husori et al., 2018). The mean time for paralysis and death was noted and the number of dead and alive was recorded.

**Collection and processing of sheep faeces**

About 60 grams of faeces were collected from the rectum of 20 sheep at the Maiduguri metropolitan abattoir into fully labeled polythene bags and brought on ice to the Parasitology Laboratory, Faculty of Veterinary Medicine, University of Maiduguri for analysis. Each faecal sample was first subjected to a simple floatation technique to ascertain the presence of helminth eggs or ova as described by Phiri et al. (2007). Positive samples were further subjected to the Modified McMasters Technique to determine the number of eggs present per gram of faeces (epg).
Only positive samples with at least 500 eggs per gram were used for the faecal culture.

**Faecal culture and harvest of larvae**

The test tube paper technique as described by Ngwese et al. (2020) was used for the faecal culture and harvest of larvae. 0.5g of each positive faecal sample was smeared on a filter paper strip and the lower end was each dipped into a test tube containing 2mls of distilled water. The test tubes were covered with a ball of clean cotton wool to stop evaporation and the set-up was allowed to stabilize for 10 days at room temperature. After 10 days, the cotton wool covering the test tubes was removed and the filter paper strip was carefully pulled out of the culture medium individually. The culture medium was then poured into a Petri dish and **Strongyle larvae** were identified as described by Phiri et al. (2007).

**In vitro anthelmintic activity assay of the extract on Strongyle larvae**

The anthelmintic activity was performed using a standard protocol as described by Ajaiyeoba et al. (2001). About 0.5ml each of the culture medium containing between 14 and 46 larvae was exposed to concentrations of 31.25, 62.5, 125 and 250 mg/ml of *B. aegyptiaca* stem bark aqueous extract as extract controls and Albendazole (Albavet®) concentrations 6.25mg/ml, 12.50 mg/ml and 25 mg/ml and distilled water as positive and normal controls. 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 alive parasites was determined by subtracting the alive from the known number of immobile larvae in each Petri dish before the commencement of the assay. **Larval mortality** was calculated using the formula described by Fernandez et al. (2009):

\[
\text{Larval mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae tested}} \times 100
\]

The mortality index was also calculated for each concentration of the extract as well as the standard drug (Albendazole) using the formula as described previously (Nasai et al., 2016).

\[
\text{Mortality index} = \frac{\text{Total number of immobile/dead larvae}}{\text{Total number of larvae per petri dish}} \times 100
\]

**Statistical analysis**

Data collected were expressed as mean ± standard deviation (S.D.). Linear regression tests and analysis of variance (ANOVA) were performed using GraphPad Prism v.5 and p< 0.05 was considered significant. Also, the IC₅₀ and IC₉₀ values were created and calculated using the software GraphPad Prism v.5 (GraphPad Software, La Jolla California, USA).

**Results**

The results of the anthelmintic activity of *B. aegyptiaca* stem bark aqueous extract described as time taken for paralysis and death of the earthworm (*P. posthuma*) in relation to the negative and positive controls is presented in Table 1. There was a statistically significant difference (p<0.05) between the extract controls and the positive control in terms of the time taken for paralysis and death of the earthworms. The time taken for both paralysis and death of the earthworms became shorter with an increase in extract concentration. **Table 2** shows the anthelmintic activity of *B. aegyptiaca* stem bark aqueous extract on mortality

**Table 1**: Anthelmintic activity of *Balanites aegyptiaca* stem bark aqueous extract on time taken for paralysis and death of *Pheretima posthuma* within 60 minutes

<table>
<thead>
<tr>
<th>Test Groups</th>
<th>Mean ± S. D (range) of minutes post exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paralysis</td>
</tr>
<tr>
<td><em>Balanites aegyptiaca</em></td>
<td></td>
</tr>
<tr>
<td>300 mg/ml</td>
<td>0a</td>
</tr>
<tr>
<td>400 mg/ml</td>
<td>22.00±1.00b (21-23)</td>
</tr>
<tr>
<td>500 mg/ml</td>
<td>14.28±1.11b (13-16)</td>
</tr>
<tr>
<td>600 mg/ml</td>
<td>12.60±2.55b (9-15)</td>
</tr>
<tr>
<td>Albendazole</td>
<td></td>
</tr>
<tr>
<td>25.00 mg/ml</td>
<td>28.00±1.00c (27-29)</td>
</tr>
<tr>
<td>12.50 mg/ml</td>
<td>36.80±1.64c (35-39)</td>
</tr>
<tr>
<td>6.25 mg/ml</td>
<td>37.40±1.40c (36-39)</td>
</tr>
<tr>
<td>Distilled water</td>
<td>0± 0a</td>
</tr>
</tbody>
</table>

Mean ± SD values within columns with different superscripts are statistically significant (p< 0.05)
pattern of *Pheretima posthuma*. There was a positive correlation between graded extract concentrations and the mean number of earthworms alive and those dead 1 hr post-exposure. The IC$_{50}$ and IC$_{90}$ values for the time taken for paralysis of the earthworms were recorded as 442.7 mg/ml and 767.80 mg/ml, respectively with a coefficient of determination ($r^2$) as 0.1814 (Figure 1). Finally, the IC$_{50}$ and IC$_{99}$ values for the time taken for death of the earthworms were 383.9 mg/ml and 755.0 mg/ml, respectively with a coefficient of determination ($r^2$) as 0.1471 (Figure 2).

Table 3 shows the anthelmintic activity of *B. aegyptiaca* stem bark aqueous extract on *Strongyle* larvae. There was a significant difference (p< 0.05) between the extract treatment groups and the positive control (Albendazole) in terms of the time taken for paralysis and death of the *Strongyle* larvae. There was a positive correlation between the graded extract concentrations and both larval mortality and mortality index. The 250 mg/ml concentration of *B. aegyptiaca* stem bark aqueous extract compared favorably with the positive control (Albendazole) having a larval mortality and mortality index of 1 and 100 respectively.

### Table 2: Anthelmintic activity of *Balanites aegyptiaca* stem bark aqueous extract on mortality pattern of *Pheretima posthuma*

<table>
<thead>
<tr>
<th>The concentration of the extract</th>
<th>Alive Mean ± S. D.</th>
<th>Dead Mean ± S. D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 mg/ml</td>
<td>5.00±0</td>
<td>0±0</td>
</tr>
<tr>
<td>400 mg/ml</td>
<td>3.50±0.70</td>
<td>1.50±0.71</td>
</tr>
<tr>
<td>500 mg/ml</td>
<td>1.50±0.71</td>
<td>3.50±0.71</td>
</tr>
<tr>
<td>600 mg/ml</td>
<td>0±0</td>
<td>5.00±0</td>
</tr>
</tbody>
</table>

### Figure 1: Regression equation of time of paralysis of earthworms against concentration

### Figure 2: Regression equation of time of death of earthworms against concentration

### Table 3: Anthelmintic activity of *Balanites aegyptiaca* stem bark aqueous extract on *Strongyle* larvae within 60 minutes

<table>
<thead>
<tr>
<th>Test Groups</th>
<th>Mean ± S. D. Alive</th>
<th>Larval mortality</th>
<th>Mortality index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Balanites aegyptiaca</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31.25 mg/ml</td>
<td>25.5±4.95$^a$</td>
<td>0.15</td>
<td>15.0</td>
</tr>
<tr>
<td>62.5 mg/ml</td>
<td>17.5±12.02$^b$</td>
<td>0.45</td>
<td>45.3</td>
</tr>
<tr>
<td>125 mg/ml</td>
<td>17±12.73$^b$</td>
<td>0.26</td>
<td>26.09</td>
</tr>
<tr>
<td>250 mg/ml</td>
<td>0±0$^c$</td>
<td>1.00</td>
<td>100.0</td>
</tr>
<tr>
<td>Albendazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.25 mg/ml</td>
<td>0±0$^c$</td>
<td>1.00</td>
<td>100.0</td>
</tr>
<tr>
<td>12.5 mg/ml</td>
<td>0±0$^c$</td>
<td>1.00</td>
<td>100.0</td>
</tr>
<tr>
<td>25 mg/ml</td>
<td>0±0$^c$</td>
<td>1.00</td>
<td>100.0</td>
</tr>
<tr>
<td>Distilled water</td>
<td>29±15.56$^a$</td>
<td>0±0$^d$</td>
<td>0</td>
</tr>
</tbody>
</table>

Mean ± SD values within column with different superscripts are statistically significantly (p< 0.05)
Discussion

Helminthosis is a serious disease in humans and in livestock farming. Although several commercial drugs are available in the market because of their side effects, medicinal plants have been appreciated as an alternative source of anthelmintic drugs. A handful of conducted studies have confirmed the effectiveness of many plants possessing anthelmintic activity. Anthelmintic drugs are known to act by causing paralysis of worms or damaging cuticles, leading to partial digestion. They interfere with the metabolism of worms since the metabolic requirements of these parasites vary greatly from one species to another (Swargiary et al., 2013).

In this study, B. aegyptiaca stem bark aqueous extract at graded concentrations tested in vitro showed significant anthelmintic activity against Strongylo larvae and the earthworm P. posthuma similar to albendazole. Studies on the therapeutic values of B. aegyptiaca have shown that the root bark has anthelmintic activity and it owes its medicinal values from the presence of phytochemicals (Dwivedi et al., 2009). Helminthes or specifically nematodes are only one level lower than earthworms, the observed anthelmintic effects of the extracts on the earthworms is a mirror of what the extract will also exhibit on the stongyle larvae (Nasai et al., 2016). This was further confirmed with the in vitro experiment of the B. aegyptiaca stem bark extract on infective Strongylo larvae, giving credence to support the use of earthworms as a model to study the anthelmintic activity of plant extracts.

To screen the anthelmintic activity of plant compounds, in vitro testing of L3 is regarded as the best approach (Hernández-Villegas et al., 2011). The larvicidal activity of the extract was directly dependent on the concentration dose of the extract with high activity at the 250 mg/mL treatment group. Our study observed that the highest larvicidal activity of B. aegyptiaca stem bark was at 250 mg/mL producing an efficacy of 100.0% for the aqueous extract. The treated larvae were immobile and the findings suggest that the phytochemicals present caused severe weakening of the larvae that eventually resulted in their death. Our observation from this study confirms the traditional applications of B. aegyptiaca against internal parasites, as plants containing phytochemicals like alkaloids and saponins, possess antiparasitic activity. These bioactive compounds work separately or jointly to alter the membrane permeability of the parasite (Chothani & Vaghasiya, 2011) or binding to a specific glycoprotein of the cuticle of the parasite (Kumar et al., 2011).

The most important are steroidal saponins, which yield diosgenin, a source of steroidal drugs, such as corticosteroids, contraceptives and sex hormones as described by Farid et al. (2002). Others include cardiac glycosides, tannins and alkaloid salts. Specific compounds that have been isolated from the stem bark of B. aegyptiaca are furanocoumarin bergapten and dihydrofuranocoumarin D- marmesin (Murthy et al., 2020), as well as three common metabolites, vanillic acid, syringic acid; and 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone and two specific alkaloids namely N-trans-feruloyltyramine and N-cis-feruloyltyramine (Ansari et al., 2006; Breimer et al., 2007). Additionally, a long-chain aliphatic compound and a novel sugar, di-glucosyl-di-rhamnoside and 10-methyl-n-heptacosane, have also been isolated from the stem barks (Kapseu et al., 1997; Hardman et al., 2001; Ansari et al., 2006; Breimer et al., 2007; Al-Thobaiti & Abu Zeid, 2018). Saponins exhibit their effects by causing damage to the membrane of the parasite as well as vacuolization and disintegration of their integument (Wang et al., 2010) while tannins are known to hinder energy production by uncoupling oxidative phosphorylation by binding to glycoproteins on the cuticle of helminths thereby leading to the death of the parasite. For instance, Balantin-7, a steroidal saponin isolated from B. aegyptiaca was shown to exhibit strong anthelmintic activity against Caenorhabditis elegans adult worm viability (Gnoula et al., 2007). Furthermore, alkaloids can intercalate with the protein synthesis of the parasite (Al-Shaibani et al., 2009). Koko et al. (2005) pointed out that the plant is used as a purgative to remove intestinal parasites with the root, branches, bark, fruit and kernel extracts shown to be lethal to the miracidia and cercariae of Schistosoma mansoni and to Fasciola gigantica.

Albendazole, a proprietary anthelmintic belongs to the benzimidazole group and acts by uncoupling mitochondrial function associated with electronic transport to enable ATP generation. The molecular mode of action of all benzimidazoles, including albendazole, consists in binding to tubulin, a structural protein of microtubules. The blocking of microtubules in the worm perturbs the uptake of glucose leading to the exhaustion of glycogen reserves. This blocks the whole energy management mechanism of the worms that are paralyzed and they die and are subsequently are expelled. Albendazole also inhibits fumarate reductase, an enzyme involved...
in the energy management of the worm cells as well (Adedapo et al., 2007).
In conclusion, the results of this study showed that the aqueous extract of *B. aegyptiaca* stem bark produced immobilization of earthworms (100.0%) and *Strongyle* larvae (100.0%) under *in vitro* conditions. Also, the promising anthelmintic activity of the extract supports its folkloric use by locals in the treatment of helminth infections in both humans and animals.

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No funding was received.

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

**References**


