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Research Article

*In vitro* anthelmintic activity of *Heliotropium indicum*, *Senna fistula* and *Spigelia anthelmia* used as worm expeller in South West Nigeria
In vitro anthelmintic activity of Heliotropium indicum, Senna fistula and Spigelia anthelmia used as worm expeller in South West Nigeria

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Abstract
The anthelmintic potential of Heliotropium indicum, Senna fistula and Spigelia anthelmia were investigated using nematodes larvae from sheep and adult earthworms in order to justify the folkloric claim of the plants as worm expeller in south west of Nigeria. The acetone, ethanol, hydro-alcohol and distilled water extracts showed dose-dependent anthelmintic activities at the different concentrations (0.3, 0.5, 1.0 mg/mL) when tested against nematodes larvae. The order of anthelmintic effect for the plants was H. indicum > S. fistula > S. anthelmia. Acetone and ethanol extracts of the three plants showed the most effective activity (100% mortality) against adult earthworm (Pheretima posthuma) after 30 min of exposure. The reference standard drug (Vermox®) showed less effectiveness compared to the medicinal plants used in the study. Overall, the study indicates that H. indicum, S. fistula and S. anthelmia are potential anthelmintic herbal drugs, which in turn validates the use of the three species as worm expellers by the Yorubas tribe of South West Nigeria.

Materials and Methods
Plant sampling
The plant materials were collected in Abeokuta North, Abeokuta South and Ewekoro local government areas of Ogun State, Nigeria. The three plant species were identified and voucher specimens lodged at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan in Nigeria.

**Preparation of extracts**

The root samples each of *H. indicum*, *S. fistula* and *S. anthelmia* were dried and ground to a fine powder. 15 g each of plant material was suspended and extracted in 150 mL of acetone, ethanol, hydro-alcohol and distilled water respectively and kept on an electric shaker at speed 100 rpm for 24 hours. The extracts were filtered using the centrifuge and the supernatants filtered through Whatman No. 1 filter paper. The acetone and ethanol extracts were concentrated using a rotary evaporator (Cole-Parmer, Shanghai Eyela Co. Ltd, China); the hydro-alcohol extracts were freeze-dried (Virtis Bench Top: SP Scientific series, USA) whereas the distilled water extracts were dried in a water bath at 45°C. The dried crude extracts were re-weighed and kept at 4°C for further use.

**In vitro anthelmintic activity**

Fecal sample obtained from the rectum of sheep were collected in autoclaved bags to prevent contamination and taken to the laboratory. Nematode eggs were incubated for 7 days at 25°C using vermiculite, after which the hatched larvae were used for the anthelminthic study. The larvae were exposed to each plant extract (acetone, ethanol, hydro-alcohol and distilled water) at varying concentrations of 0.3, 0.5 and 1.0 mg/mL. For each plant sample four petri dishes, three for each extract concentrations and one for the control (Vermox®) were used. Larval count was conducted first after 30 min then after 1 hour to record the mortality rate.

**Anthelmintic activity using Indian earthworms (Pheretima posthuma)**

The assay was conducted following the method described by Ajaiyeoba et al. (2001), and Ashok Kumar et al. (2010), with slight modification. The study was performed by using adult Indian earthworms due to its physiological and anatomical resemblance to the human intestinal roundworm (*Ascaris lumbricoides*) para-site (Chatterjee, 1967; Vidyarthi, 1967). Four worms were placed in vials containing acetone, ethanol, hydro-alcohol and water extracts of *H. indicum*, *S. fistula* and *S. anthelmia* dissolved in distilled water at different concentrations of 0.3, 0.5 and 1.0 mg/mL. The time of death was observed and recorded after the worms were vigorously shaken and observed for no movement. Vermox® was used as positive control while distilled water served as negative control. Experiment was carried out in triplicate.

**Results**

All the three concentrations of acetone, ethanol, hydro-alcohol and water extract (0.3, 0.5 and 1.0 mg/mL) of the three plants showed potent and better anthelmintic activity compared to positive control. The results are presented in Table I.

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Conc. (mg/L)</th>
<th>Percentage mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vermox 30 min 1 hour</td>
</tr>
<tr>
<td><em>Heliotropium indicum</em></td>
<td>0.25</td>
<td>0 5 90 100</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0 5 100</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>5 10 100</td>
</tr>
<tr>
<td><em>Senna fistula</em></td>
<td>0.25</td>
<td>0 5 90</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0 5 100</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>5 10 100</td>
</tr>
<tr>
<td><em>Spigelia anthelmia</em></td>
<td>0.25</td>
<td>0 5 100</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0 5 100</td>
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<tr>
<td></td>
<td>1.0</td>
<td>5 10 100</td>
</tr>
</tbody>
</table>
activity than Vermox®. There was 100% mortality rate at higher concentrations (1 mg/mL) of each plant used. At 30 min, the 0.3 mg/mL extracts of the three plants exhibited 55–100% mortality on the larvae while at 0.5 mg/mL, 70–100% mortality was recorded (Table I).

Acetone, ethanol and hydro-alcohol extracts of the three plants showed 100% effectiveness as potential anthelmintic at 0.5 mg/mL concentration at 1 hour of observation. At 1 hour, the water extract of *H. indicum* and *S. fistula* showed better anthelmintic property compared to *S. anthelmia* having mortality rate of (80, 90, 100%), (90, 95, 100%) and (70, 85, 90%) respectively.

The earthworms selected for the anthelmintic activity were most sensitive to acetone and ethanol root extracts of all the three plants (Table II). There was 100% mortality at all the three concentrations at a minimum of 30 min of exposure. *H. indicum* exhibited better anthelmintic activity with the hydro-alcohol extract than those of *S. fistula* and *S. anthelmia*. There was no death observed at lower concentrations (0.3 and 0.5 mg/mL) in all the three plant water extracts for all the time(s) the test was carried out. At 1.0 mg/mL, *H. indicum* had 50% mortality on the earthworms while it was 75% for both *S. fistula* and *S. anthelmia* respectively at this concentration which indicated that significant anthelmintic activity is concentration dependent. 25% mortality was only observed at 1.0 mg/mL after 3 hours in the positive control (Vermox®) while all the earthworms in distilled water (negative control) were living and active after 3 hours.

There was a significant difference ($F_{2,3}= 11, p<0.05$) between the plants at the lowest concentration of 0.3 mg/mL used in the study (Figure 1A). There was no anthelmintic activity observed at the least concentration of *S. anthelmia* hydro-alcohol plant extract and from distilled water extract of the three plants against the earthworms. At the 0.5 mg/mL, there was also a significant difference ($F_{2,3}= 17.4, p<0.05$) observed for the three plants (Figure 1B) and this significant difference ($F_{2,3}= 6.1, p<0.05$) was equally observed at the highest concentration (1.0 mg/mL) used in the study (Figure 1C). *S. anthelmia* showed the least effect with hydro-alcohol extract while *H. indicum* was observed to have the least anthelmintic activity with the distilled water extract.

**Discussion**

The result from the anthelmintic activity suggests that all the three plants used for the study are potent worm expeller and this agrees with the work of Sofowora, (2006) that ethno-medicinal prescription of plant for worm eradication had achieved great success with almost 63% of tropical plants showing anthelmintic properties.

The anthelmintic activity of the three plants carried out using gastrointestinal nematode larvae of sheep showed a 100% death rate in ethanol and acetone after 30 min of observation at 0.5 mg/mL concentration while for the hydro-alcohol and distilled water extracts this mortality was observed at the concentration 1.0 mg/mL also after 30 min. This confirms the folkloric claim that the plants are more effective when soaked into the local gin (more or less alcohol) to kill/expel intestinal worms, this is also in agreement with Eloff, (1998) that the isolation of botanical compounds from plant materials largely depends on the medium or solvent and method of extraction.

Earthworms have been widely used for *in vitro* initial evaluation of anthelmintic compounds due to its easy...
availability (Sollmann, 1918; Sundeen Kumar et al., 2010). The three plants showed 100% mortality in both acetone and ethanol extract for the times (30 min - 3 hours) of observation. It can, therefore, be inferred that ethanol and acetone were able to extract more of the constituents that is required to kill the worms, this result exhibited the same pattern with the work of Chavan et al. (2010), where the in vitro anthelmintic activity of the fruit extract of Barlecia prioritis was tested against adult earthworm and a better result was observed with the ethanol extract over the water extract at time 7.1 min of observation. In hydro-alcohol extracts, H. indicum was observed to be most effective while in distilled water extracts, S. anthelia showed the highest effect against the Indian earthworms. The effect of the anthelmintic activity of S. anthelia root is consistent with the report of Ezike et al. (2013), in which the whole plant methanol extract used against earthworm showed best potential anthelmintic effect of the three plants used for the study, he further claimed that the effect could be accounted for due to its alkaloids constituent as such compounds have been associated with anthelmintic activity (Makkaret al., 2007). H. indicum has been proven by Fu et al. (2002), to contain tumorigenic pyrrolizidine alkaloids (normal cell that are transformed into cancer cells) and should not be consumed in larger quantity. The vermicidal activity of the extracts against earthworms suggests that these plants would be effective against human intestinal parasitic infections.

### Conclusion

The use of acetone, ethanol and hydro-alcohol extracts are recommended for better effect of anthelmintic activity of H. indicum, S. fistula and S. anthelia as there was less or almost no effect was observed with the use of distilled water extract. H. indicum and S. fistula are recommended to be used as human intestinal worm expeller instead of S. anthelia which is widely known in Nigeria.

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### Conflict of Interest

Authors declare no conflict of interest

### Acknowledgement

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### References


Eloff JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J Ethnopharmacol. 1998; 60: 1-8.


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Table II

Effect of Vermox®, Heliotropium indicum, Senna fistula and Spigelia anthelminia root extracts on earthworms (Pheretima posthuma)

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Conc. (mg/mL)</th>
<th>Control</th>
<th>% mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vermox</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Heliotropium indicum</td>
<td>0.25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>1.0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Senna fistula</td>
<td>0.25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0</td>
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<tr>
<td></td>
<td>1.0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Spigelia anthelminia</td>
<td>0.25</td>
<td>0</td>
<td>0</td>
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<td></td>
<td>0.5</td>
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<td>1.0</td>
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</tbody>
</table>

*All earthworm died within 30 min. **All earthworm died within 1 hour