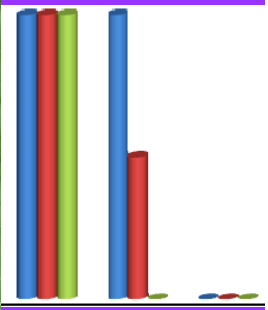

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In vitro anthelmintic activity of *Heliotropium indicum, Senna fistula* and *Spigelia anthelmia* used as worm expeller in South West Nigeria

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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 earth worm (*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.

Introduction

Trichiuris trichiuria, Ascaris lumbricoides and *Ancylostoma duodenale* have been reported to be most prevalent in developing world where there is poor sanitation facilities and inadequate water supply (Savioli, 2004). In Africa, intestinal cestode infection is of great concern and it is closely linked with poor environmental hygiene and poverty (Carabinet al., 2006; Abunnaet al., 2007). Intestinal parasites have been long recognised among Nigerian children between the ages 0-18 years as a major health problem (Ajayi and Akinyinka, 1999; Oguoma et al., 2008), in which majority of the children were found to be stunted with retarded growth, anaemic and underweight due to malnutrition.

Heliotropium indicum is highly valued in the folklore medicine in the treatment of different diseases (Muthuet al., 2006; Togolaet al., 2005). *Senna fistula* is used as purgative having active ingredients anthraquinone derivates and their glycosides. It is also used as a laxative acting on the lower bowel especially in alleviating constipation (Spiller et al., 2003). *Spigelia anthelmia* L. is known as 'Aparan' or 'ewe aran' (Dalziel, 1937), among the Yoruba tribe of south western Nigeria. According to Ezikeet al. (2013), the native inhabitants of south western Nigeria typically use *S. anthelmia* for expelling worms.

Chemotherapy is used to control helmintic infection in Nigeria; however the drugs are not readily available to the dwellers of the aforementioned areas (Mbaria et al., 1998). This study presents an alternative approach for treating intestinal worms in order to validate the folkloric claims about these plants as worm expellers.

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. indi-cum, 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

Table I

Effect of root extracts of *H. indicum, S. fistula and S. anthelmia* on larvae mortality of gastrointestinal nematode of livestock

Test sample	Conc. (mg/L)	Percentage mortality									
		Vermox		Acetone extract		Ethanol extract		Hydroalcohol extract		Distilled water	
		30 min	1 hour	30 min	1 hour	30 min	1 hour	30 min	1 hour	30 min	1 hour
Heliotropium indicum	0.25	0	5	90	100	95	100	60	95	65	80
	0.5	0	5	100	100	100	100	90	100	70	90
	1.0	5	10	100	100	100	100	100	100	90	100
Senna fistula	0.25	0	5	90	100	100	100	80	90	65	90
	0.5	0	5	100	100	100	100	90	100	75	95
	1.0	5	10	100	100	100	100	100	100	90	100
Spigelia anthelmia	0.25	0	5	100	100	100	100	70	95	55	70
	0.5	0	5	100	100	100	100	90	100	70	85
	1.0	5	10	100	100	100	100	100	100	80	90

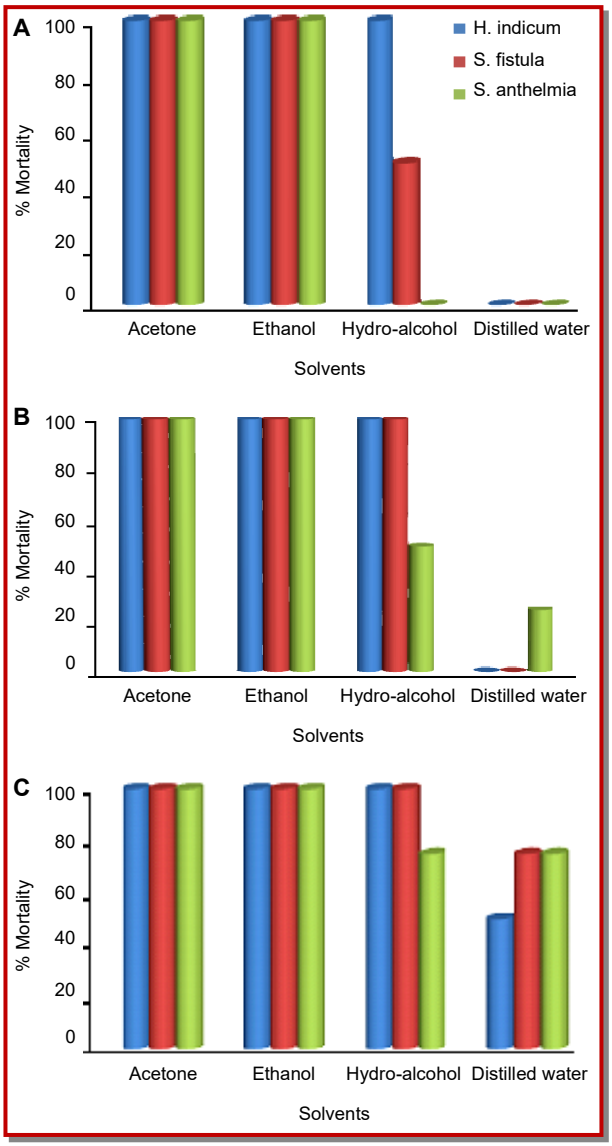

Figure 1: Comparative percentage mortality of 0.25 mg/mL (A), 0.5 mg/mL (B) and 1.0 mg/mL (C) of the extracts against adult earth worm (*Pheretima posthum*)

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 anthelminthic 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; Sundeep 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 *Barleeia prionitis* 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. anthelmia* showed the highest effect against the Indian earthworms. The effect of the anthelmintic activity of *S. anthelmia* 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 anthelminthic activity of *H. indicum, S. fistula* and *S. anthelmia* 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. anthelmia* which is widely known in Nigeria.

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Conflict of Interest

Authors declare no conflict of interest

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Table II

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

Test sample	Conc. (mg/mL)	Control	% mortality																		
			Vermox				Acetone extract				Ethanol extract				Hydro-alcohol extract				Distilled water			
		30 min	1 hour	2 hours	3 hours	30 min	1 hour	2 hours	3 hours	30 min	1 hour	2 hours	3 hours	30 min	1 hour	2 hours	3 hours	30 min	1 hour	2 hours	3 hours
Heliotropium indicum	0.25	0	0	0	0	100	a	a	a	100	a	a	a	75	100	b	b	0	0	0	0
	0.5	0	0	0	0	100	a	a	a	100	a	a	a	100	a	a	a	0	0	0	0
	1.0	0	0	0	25	100	a	a	a	100	a	a	a	100	a	a	a	0	25	50	50
Senna fistula	0.25	0	0	0	0	100	a	a	a	100	a	a	a	0	0	25	50	0	0	0	0
	0.5	0	0	0	0	100	a	a	a	100	a	a	a	50	50	75	100	0	0	0	0
	1.0	0	0	0	25	100	a	a	a	100	a	a	a	100	a	a	a	0	50	50	75
Spigelia anthelmia	0.25	0	0	0	0	100	a	a	a	100	a	a	a	0	0	0	0	0	0	0	0
	0.5	0	0	0	0	100	a	a	a	100	a	a	a	0	0	25	50	0	0	0	25
	1.0	0	0	0	25	100	a	a	a	100	a	a	a	25	25	50	75	0	25	50	75

[a]All earthworm died within 30 min. [b]All earthworm died within 1 hour