

Preliminary Phytochemical Screening and *in vitro* Anthelmintic Effects of Aqueous Extracts of *Salvadora persica* and *Terminalia avicennoides* Against Strongyline Nematodes of Small Ruminants in Nigeria

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Abstract: The traditional use of aqueous extracts of shoots and leaves of *Salvadora persica* L. and root bark of *Terminalia avicennoides* as anthelmintics in Northeastern Nigeria necessitated the need to examine these plants. This study was carried out to determine the phytochemical constituents of these extracts and determine and compared their anthelmintic efficacies against those of commercially available anthelmintics (albendazole and levamisole). The preliminary phytochemical screening of the extracts revealed the presence of tannins, flavonoids, saponins, sterols and terpenes and reducing sugars in all the extracts. Anthracenocides were present only in extracts of *S. persica* L. shoots and the root bark of *T. avicennoides*. Similarly, flavone aglycones were detected only in extracts of *S. persica* L. leaves and the root bark of *T. avicennoides*. Basic alkaloids were detected in extracts of *T. avicennoides* stem bark and *S. persica* shoots. The anthelmintic study showed that all the extracts exhibit *in vitro* anthelmintic activities against strongyline nematodes in a concentration dependent fashion and such effects were significant ($p < 0.001$) when compared to those of albendazole and levamisole at the concentrations used in the experiment. The result of the study indicated that the extracts contained phytochemicals that are known to possess various pharmacologic activities and of therapeutic benefits, one or a combination of which could be responsible for the *in vitro* anthelmintic effects observed. The study also validated the basis for the traditional use of these plants as anthelmintics in North Eastern Nigeria.

Key words: Phytochemicals, *Salvadora persica*, *Terminalia avicennoides*, anthelmintic, small ruminants, nematodes

INTRODUCTION

Helminthosis is the most common economically important infectious disease of grazing livestock especially small ruminants in the tropics and subtropics (Githiori *et al.*, 2003a). The disease is characterized by high morbidity (Suleiman *et al.*, 2005) and causes wastage and serious economic loss in small ruminant production thereby constituting a major constraint in the development of these species (Nawathe *et al.*, 1985; Payne, 1990). It is also the major cause of mortality and sub-optimal productivity in these species especially under the traditional husbandry system (Fakae, 1990; Kusiluka and Kambarage, 1996). Losses in production due to helminthosis is either direct due to mortalities or indirect as a result of reduction in live-weight gains,

decreased quality of skin, wool or mohair and diminished immune status of infested animals leading to increased susceptibility to other infections (Kusiluka and Kambarage, 1996). Reduced growth rate (McLeod, 1995) and decrease milk yield (Mulugeta *et al.*, 1987) are also common findings associated with the disease.

Control of helminthosis is usually aimed at reducing its prevalence and limiting infection in order to minimize the challenge to an economically justifiable level. This is usually achieved through judicious use of anthelmintics, good management, breeding resistant stock and immunization (Kusiluka and Kambarage, 1996). However, the use of anthelmintics which is foremost among the control measures faces serious setback as a result of the emergence and spread of drug-resistant gastrointestinal parasites (Prichard, 1994; Mwamachi *et al.*, 1995; Waller,

1997). This has been blamed on the misuse of anthelmintic drugs and probable adaptation of parasites to commercially available formulations (Mascie-Taylor and Karim, 2003) and the widespread intensive use of low quality anthelmintics (Monteiro *et al.*, 1998). Furthermore, commercially available anthelmintics are relatively expensive (Hammond *et al.*, 1997) and smallholder farmers are unlikely to spend their meager income to purchase these drugs for regular treatments (Kusiluka and Kambarage, 1996). This necessitates the search for alternative chemotherapeutic agents that are relatively inexpensive, readily available and to which parasites are unlikely to develop resistance (Coles and Roush, 1992; Hammond *et al.*, 1997).

Materials from plants have been used in many parts of the world as traditional remedies against helminths that affect humans and animals and quite a number of them have been scientifically validated for their anthelmintic properties (Githiori *et al.*, 2003a, b; Adedapo *et al.*, 2005; Dawo and Tibbo, 2005; Suleiman *et al.*, 2005; Max *et al.*, 2007; Jan *et al.*, 2008).

In northeastern part of Nigeria, the aqueous extracts of the leaves and shoots of *Salvadora persica* L., a member of the Salvadoraceae family and the root bark of *Terminalia avicennoides* of the family Combrataceae are used in traditional medicine for the treatment of a myriad of diseases including helminthes infections. Therefore, given the wide application of these plants in the traditional treatment of various conditions and as anthelmintics, there is the need to determine the chemical constituents and validate the claim for their activities.

The aim of this study was to determine the preliminary phytochemical constituents of the aqueous extracts of the shoots and leaves of *Salvadora persica* L. and root bark of *Terminalia avicennoides* and to determine and compared their anthelmintic efficacies against those of commercially available anthelmintics (albendazole and levamisole).

MATERIALS AND METHODS

Study area: The study was carried out in Borno state in the Northeastern Nigeria, located within latitude 10°N and 14°N and longitude 11°30'-14°45'E.

Collection and identification of plant materials: Fresh leaves and shoots of *Salvadora persica* L. and the root bark of *Terminalia avicennoides* were collected from neighboring townships, Monguno and Konduga, respective in Borno state of Nigeria in August 2008. The identities of the plants were authenticated by a plant taxonomist in the Department of Biological Sciences,

University of Maiduguri, Borno state. Voucher specimens (No. 545B) were deposited in the laboratory of the Department of Chemistry of the same institution where the preliminary phytochemical analyses were done. Each of the collected plant materials were separately air-dried, pulverized and kept in cellophane bags before extraction.

Preparation of plant extracts: About 200 g each of the powdered samples of the shoots and leaves of *S. persica* L. and root bark of *T. avicennoides* were separately subjected to exhaustive soxhlet extraction in 500 mL of distilled water for 6 h. The extracts were then concentrated *in vacuo* in a rotary evaporator. There were then labeled and stored at 4°C until use (Trease and Evans, 2002).

Phytochemical screening: The different aqueous extract fractions of the shoots and leaves of *S. persica* L. and root bark of *T. avicennoides* were separately subjected to preliminary phytochemical screening using standard procedures (Harborne, 1993; Trease and Evans, 2002).

Collection and identification of nematode eggs: Fecal samples were collected directly from the rectum of 30 sheep and goats of different breeds randomly selected during slaughter at the Maiduguri Metropolitan Abattoir. The samples were placed in polythene bags and transported to the laboratory for analysis. Egg counts were determined by the McMaster technique using saturated sodium chloride solution as the floating medium (Gordon and Whitlock, 1939; Nwosu *et al.*, 2001). Only fecal samples with at least 500 Eggs Per Gramme (EPG) were used in the study.

Fecal culture and laval recovery: These were done using the test tube filter paper technique as described by Harada and Mori (1955). Identification of infective nematode larvae was done based on standard descriptions (Hansen and Perry, 1990, 1994; Anonymous, 1977).

Anthelmintic assay: The anthelmintic efficacy of the extracts at 25, 50 and 100 mg mL⁻¹ concentrations were determined by egg hatch assay as described by Kelly *et al.* (1981). Two commercially available anthelmintics, albendazole and levamisole (as positive control) were compared with the extracts for their efficacy in preventing egg hatch. Tap water was used as negative control. The number of eggs hatched was determined for the extract-treated and control culture samples and were then compared to the unhatched eggs to determine the percentage reduction in egg hatch. The proportion of

unhatched eggs at each concentration of the extracts and drugs used were calculated by relating the number of hatched larvae to the total number of eggs cultured (Chiejina, 1984).

Statistical analysis: Data were analysed using computer statistical software package, Graphpad instat version 3.10, 32 bit for Windows, 2009 by GraphPad software inc. The reduction in egg hatch in fecal cultures treated with the plant extracts and those of the control groups were expressed as mean and standard deviation. Analysis of Variance (ANOVA) was used to compute the differences among means and $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Phytochemical analysis: The result of the phytochemical screening of *Salvadora persica* L. shoots and leaves extracts and *Terminalia avicennoides* root bark extract are shown in Table 1. The result revealed slight to moderate presence of sterols and terpenes, flavonoids, saponins, tannins and reducing sugars in all the extracts. Anthracenocides are slightly present in *S. persica* L. shoots extract but absent in its leaves and the root bark of *T. avicennoides*. Slight to moderate presence of flavone aglycone were detected in *S. persica* L. leaves and the root bark of *T. avicennoides* but absent in the shoots of *S. persica* L. Basic alkaloids were also moderately present in *T. avicennoides* stem bark and *S. persica* L. shoots but absent in the latter's leaves.

Anthelmintic assay: The result of *in vitro* anthelmintic efficacy of the aqueous extracts of *S. persica* L. and *T. avicennoides* compared to those of the control groups are shown in Table 2 and 3, respectively. The percentage reductions in egg hatch were found to be concentration dependent as the highest dilution of the extracts produced the least inhibition in egg hatch and vice versa and these effects were significantly ($p < 0.001$) similar to the result of positive controls albendazole and levamisole although at the extracts' concentrations of 50 and 25 mg mL⁻¹, the reductions in egg hatch were slightly less than those of the standard drugs.

The result of the phytochemical screening of aqueous extracts of shoots and leaves of *Salvadora persica* L. and the root bark of *Terminalia avicennoides* revealed the presence of phytochemicals that are known to possess various pharmacologic activities and of therapeutic benefits. The result also showed that the extracts exhibit *in vitro* anthelmintic properties against strongyline nematodes. Studies on other plants have revealed that phytochemicals similar to those present in the extracts used in this study has anthelmintic activity.

Table 1: Phytochemical constituents of *Salvadora persica* and *Terminalia avicennoides*

<i>Salvadora persica</i> / <i>Terminalia avicennoides</i>			
Phytochemicals	SP _L	SP _S	TA _{RB}
Sterols/terpenes	++	+	+
Reducing components	+	+	+
Flavone aglycone	++	-	+
Anthracenocides	-	+	-
Flavonoids	++	+	+
Saponins	+	++	++
Tannins	+	+	+
Basic alkaloids	-	+	+

++ = Moderate presence, SP_L = *Salvadora persica* leaf, + = Slight presence, SP_S = *Salvadora persica* shoot, - = Absence and TA_{RB} = *Terminalia avicennoides* root bark

Table 2: *In vitro* efficacy of the aqueous extract of *Salvadora persica* (shoots and leaves) against strongyline nematodes

Extract/drug	Concentration (mg mL ⁻¹)	No. of larvae hatched (Mean±SD*)	Percentage reduction in egg recovery (%)
Water control	-	185.28±44.11	0.0**
SP _A	100.00	1.28±1.980	98.9
	50.00	2.57±1.740	98.9
	25.00	18.64±5.410	86.7
SP _B	100.00	1.42±1.700	99.9
	50.00	2.79±1.360	98.3
	25.00	18.27±4.720	72.4
Albendazole	25.00	0	100.0
	12.50	1.79±0.800	99.5
	6.25	7.00±3.890	98.3
Levamisole	60.00	0	100.0
	30.00	2.0±4.7700	98.9
	15.00	8.9±4.7300	93.4

*Mean standard deviation based on 10 observations, **Larval recovery for control (distilled water) culture used as standard reduction in egg hatch, SP_A = *Salvadora persica* shoot extract, SP_B = *Salvadora persica* leaf extract reductions (Mean±SD) in the no. of larvae hatched in the extract treated groups are significant ($p < 0.001$) when compared to those of levamisole and albendazole (positive control)

Table 3: *In vitro* efficacy of the aqueous extract of *Terminalia avicennoides* (root bark) against strongyline nematodes

Extract/drug	Concentration (mg mL ⁻¹)	No. of larvae hatched (Mean±SD*)	Percentage reduction in egg recovery (%)
Water control	-	114.7±16.25	0.000**
TA _{RB}	100.00	0.00±0.00	100.000
	50.00	18.50±2.31	83.700
	25.00	32.55±3.37	71.580
Albendazole	25.00	0.00±0.00	100.000
	12.50	13.35±2.52	88.320
	6.25	28.60±2.58	75.070
Levamisole	60.00	0.00±0.00	100.000
	30.00	12.50±2.33	89.100
	15.00	26.00±2.73	77.310

*Mean standard deviation based on 10 observations, **Larval recovery for control (distilled water) culture used as standard (% reduction in egg hatch). TA_{RB} = *Terminalia avicennoides* root bark extract. Reductions (Mean±SD) in the no. of larvae hatched in the extract treated groups are significant ($p < 0.001$) when compared to those of levamisole and albendazole (positive control)

Tannins are polyphenolic compounds (Min *et al.*, 2004; Ramirez-Restrepo *et al.*, 2004; Makkar *et al.*, 2007; Max *et al.*, 2007) that have been reported to have profound anthelmintic effects (Butter *et al.*, 2000;

Athanasiadou *et al.*, 2000, 2001; Max *et al.*, 2005a, b, 2007). Reports on the mechanism by which tannins produce anthelmintic effect remain equivocal. Action similar to those of synthetic phenolic anthelmintics such as oxyclozanide, niclosamide and nitroxylnil which interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation, consequently leading to depletion of parasite ATP has been reported (Martin, 1997). Other reports suggested tannins' ability to bind to free proteins in the gastrointestinal tract of host animal (Niezen *et al.*, 1993; Wang *et al.*, 1996; Athanasiadou *et al.*, 2001) or to glycoproteins on the cuticle of parasites (Thompson and Geary, 1995), leading to death of the parasites. A form of terpene known as palasonin (a terpene anhydride) was reported to produce anthelmintic activity by inhibiting glucose uptake and depleting the glycogen content of parasites (Kumar *et al.*, 1995). Similarly, gentistein (a form of flavone aglycone) was reported to produce anthelmintic effect which was linked to the possibly of its activity on nitric oxide synthetase (Kar *et al.*, 2002). The anthelmintic effects of flavonoids were also reported (Lahlou, 2002; Trease and Evans, 2002).

Villasenor *et al.* (1998) and Cho *et al.* (2003) observed that better therapeutic effect may be obtained from a combination of active principles in each plant than by single isolated substance. Therefore, the individual or collective presence of the phytochemicals discussed above in the extracts used in this study may possibly constitute the basis for the profound anthelmintic activity exhibited by the extracts.

This study also revealed that the anthelmintic effects of all the extracts studied were concentration dependent which indicates that the extract exhibit graded anthelmintic efficacy. Reductions (Mean±SD) in hatched larvae in all the extract treated groups are significant ($p<0.001$) when compared to those of the positive control group (albendazole and levamisole). This result holds a potential promise in the future use of materials from these plants as anthelmintics.

CONCLUSION

It is therefore concluded from this study that the shoots and leaves extracts of *S. persica* and root bark extract of *T. avicennoides* contain phytochemicals of therapeutic significance and have anthelmintic properties with proven efficacy against strongyline gastrointestinal nematodes. This finding has validated the basis for the traditional use of these plants against gastrointestinal helminthes in northeastern Nigeria. Researcher therefore recommend further bioassay guided fractionation and

phytochemical analysis of the constituents of these extracts with the aim of possibly isolating the anthelmintic constituents.

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