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### Anthelmintic Efficacy Trials using Fractionsof Ethanolic Crude Extract of Anogeissusschimperi Hoechst against Nippostrongylus braziliensis in Rats.

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ABSTRACT

This study screened three fractions obtained through Bioassay guided separation of constituents ofAnogeissus schimperi Hoechst; butanol (BF), ethylacetate (EF) and aqueous (AF) each at 50 mg kg<sup>-1</sup> body weight for anthelmintic activity against experimentally infected Nippostrongylus braziliensis in rats so as to establishing which of the fractions contained anthelmintic properties as a prelude to actual anthelmintic studies. The butanol, ethylacetate and aqueous fractions gave percentage anthelmintic activity of 28.8%, 53.33% and 62.22%, respectively. The aqueous and ethyl acetate fractions gave a highly significant (P<0.05) activity, while the butanolfraction gave a non-significant (P>0.05) activity when compared with the control group. When compared also with the crude extract, the aqueous fraction gave a significant activity. The aqueous fraction was therefore found to be the most active anthelmintic fraction, an indication of the probable presence of water - soluble active anthelmintic principle(s) in Anogeissus schimperi.

**Key word:** fractions, activity, anthelmintic, *Anogeissus schimperi, Nippostrongylus braziliensis* 

#### **INTRODUCTION**

Most of the developing countries in the world lie in the tropical and subtropical regions. The warm and humid climatic conditions in these regions provide favourable environment for development of worm eggs to infective larvae almost throughout the year. Thus, apart from nutrition, poor management and infectious diseases, helminthosis is a problem and a major limiting factor of livestock production, increased costs of management and treatment, and mortality in severe cases (Barger and Cox, 1984; Larsen et al., 1995; Hounzangbe-Adote et al., 2005; Vanderlei et al., 2014). Smallholder farmers may not easily notice effects of internal parasites on the performance of their animals because of the sub-clinical or chronic nature of thediseases they cause, which often do not result in mortality. While poor nutrition is considered the most critical factor, parasitism also constitutes great economic losses (Akerejola et al., 1979; ILCA, 1979; Okon, 1980; Davendra, 1981; Bakunzi and Serumaga-Zake, 2000).

The significant feature of helminthoses is not necessarily the acute syndrome characteristically associated with the disease, but the fact that a few hundred worms persisting over a long period could produce chronic anaemia and ultimately loss of condition and death in animals, especially if they are grazing on low quality pasture (Allonby and Urguhart, 1975). Nematode infections cause clinical disease, mortalities and reduced production. Some of these effects include impairment of the normal physiological behavior of the animals and reduced feed intake and nitrogen retention leading to decreased efficiency of utilization of feed which causes decreased performance in terms of reduced growth rates by up to 30 % or more (Lewis, 1975; Adu and Buvanendra, 1982; Provost, 1989). Other effects include low fertility of ewes and cows, low birth weight and reduced weight gain of lambs and calves, reduced milk and wool production, and decrease in the percentage of ewes rearing lambs to weaning (Johnstone et al., 1979; Meyers, 1991; Agei, 1993; Githigia et al., 1995). The insidious effects of chronic helminthosis have important implications for the attainment of maximum productivity in livestock (Chiezey, 1998). For instance, in Ethiopia Mulugeta et al. (1987) reported that each dairy cow treated against sub-clinical helminth parasite infection produced 0.60 kg milk per day more than non-treated cow. In general, economic losses due to sub-clinical infections are much more than those from clinical infections. Helminthosis has been identified as one of the greatest single impediments to the development of sheep and goats production in the tropics (Waruiri et al., 1995). In Kenya, condemnations due to helminth parasites constituted 11.8% of the total slaughter for cattle and 46.0% for sheep and goats (Githigia et al., 1995).

Current control methods for internal parasites outside Africa focus on reducing contamination of pastures through anthelmintic treatment and/or controlled grazing. In Africa, these methods are limited by high cost of anthelmintics, their uncertain availability in the rural areas where the bulk of the livestock holdings are kept, under/over dosing by stock raisers due to lack of understanding of the manufacturers' instruction or due to lack of money or both; and increased frequency of drug resistance and limited scope in many commercial pastoral systems for controlled grazing (Mathias et al., 1998). In addition, commercial anthelmintics available in the market are usually packaged for large number of animals (50 - 100 heads) (Mathias et al., 1998), which is more than the average number of animal property in each family. Thus, the major control measure against helminthosis in Nigeria is chemotherapy. However, the general availability of drugs varies and some drugs of choice are not always available. This calls for studies aimed at developing alternative approaches to control internal parasites, including exploring the efficacy of herbs used traditionally as anthelmintics. There is a long tradition of ethno-veterinary remedies and practices for the common animal diseases including gastro-intestinal (GIT) parasite infections. The significance of helminthosis has been recognized from the earliest times by local people and herdsmen who have made various attempts of control through the use of herbs.

Pastoral Fulanis in Nigeria recognize animal helminthosis to be a very serious problem in calves of less than one year old and as such a routine herbal treatment is started within a week of birth (Ibrahim *et al.*, 1983). Such herbs are easily accessible and could be cost - effective. The cost of treatment with alternative traditional methods is negligible when compared with the cost of conventional drugs (Anjaria, 1986). In addition to being very cheap, alternative herbal preparations have good nutritional value (Okon, 1980; Ibrahim, 1984; Mbaria *et al.*, 1998).

Medicinal plants are a small but important part of the biological heritage of the earth. Traditional society places a high value on this heritage, which is expressed through intimate relationship with nature. It is an undeniable fact that in today's world, herbal medicine plays a vital role in the health care for large sections of the population, especially in developing countries, where in many cases they bridge the gap between the availability of, and demand for modern medicine (Akerele, 1990). A system based on clinical usage may be more straight forward for the thoroughly studied allopathic drugs used in western medicine but difficulties can arise for plants used in traditional medicine because of the often numerous conditions for which any one drug may be employed (Evans, 1989).

The chief medicinal use of *Anogeissus* schimperi is as a vermifuge, especially for tapeworm of the horse and donkey; the bark is used, but more often the seeds, either as a remedy or as a preventive, given with guineacorn or with water in which the corn has been steeped for some time (Dalziel, 1937).

This study screened three fractions of *Anogeissus schimperi* Hoechst for anthelmintic activity against experimentally infected *Nippostrongylus braziliensis* in rats as a prelude to establishing which of the fractions contained anthelmintic properties.

#### MATERIALS and METHODS Plant collection, identification and preparation.

The bark of plant was collected from Gyelesu area of Zaria, North Western Nigeria. Taxonomic identification established by a botanist in the Department of Biological Science, Faculty of Science, Ahmadu Bello University, Zaria-Nigeria. The plant was authenticated by a comparison with the herbarium sample at the hebarium of the Department of Biological Science. Voucher specimens of the samples were deposited at the Botany Laboratory of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

The stem bark of *A. schimperi* was sun dried and pulverized into powder using laboratory mortar and pestle; after which, the material was weighed, kept in clean containers and properly labeled.The powdered bark of *Anogeissus schimperi* was extracted continuously with 95% v/v ethanol in a Soxhletapparatus. The extract from 1.3kg of plant material was evaporated to dryness to yield a residue subsequently referred to as CE (Crude extract). The solid extract (crude extract) obtained was removed and stored in labeled beakers at 4°C until required.

## Phytochemical studies of the crude ethanol extract of *Anogeis susschimperi*

The chemical constituents present in the bark of *Anogeissus schimperi*, were analysed by subjecting quantities of crude ethanol extract of the plant to physico-chemical tests. Alkaloid was tested according to the method described by Brain and Turner, 1975. While flavonoid, Tannins, saponin and carbohydrate were tested according to the methods described by Trease and Evans respectively.

#### Bioassay guided separation of constituents of Annogeissusschimperi

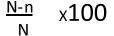
20g of the extract was weighed and dissolved in aqueous 30% v/v ethanol. The solution was filtered with cotton wool, fixed to a funnel to remove the residue formed (debris). The extract solution was then collected in a beaker. The extract solution (filtrate) was partitioned with petroleum ether 200mls; this was repeated four times giving a total of 800mls of petroleum ether being used for the partitioning. The two fractions were then collected in two separate beakers beginning with the extract fraction. After partitioning with petroleum ether, the extract solution was then partitioned with ethyl acetate 200mls this was also repeated four times giving a total of 800mls of ethyl acetate being used for the partitioning. The two fractions were then collected in two separate beakers beginning with the extract fraction. The same procedure was repeated for butanol 200mls this was repeated four times giving a total of 800mls of butanol being used for the partitioning. The two fractions were then collected in two separate beakers beginning with the extract fraction. The filtrates collected from the various fractions of partitioning above were evaporated to dryness on a steam bath. The dried solid extracts obtained were removed and stored in labeled bottles and kept until required. The separation chart for the partitioning is as shown below in Fig. 1.

# Anthelmintic efficacy trials using fractions of ethanol crude extract of *Anogeissus schimperi*.

The three fractions, Aqueous (AF), Butanol (BF) and Ethylacetate (EF) of the crude extract obtained through partitioning were screened for anthelmintic activity. This was carried out to determine the most active fraction.

The anthelmintic screening was done as described by Cavier (1973). The screening was carried out using the method described by Cavier(1973). Twenty five Albino rats (Wistar strain) weighing between 80~200g.All the rats were dewormed using albendazole (Concept Pharmaceuticals, India) at 7.5 mg/kg in order to establish a worm-free colony. The rats were identified by marks on their tails and cages. They were screened on 7<sup>th</sup> day after infecting

each of 25 rats with 200 larvae (L3) of *Nippostrongylus brazilliensis* and grouped into five groups of five rats each. On the 8<sup>th</sup> day post-infection, first three groups of five rats each were used to test a dose of 50 mg kg<sup>-1</sup> body weight for each of the three fractions. The fourth grouped were administered distilled water at 5 ml kg<sup>-1</sup> body weight served as negative control while the fifth group of five rats were given Albendazole at 7.5 mg kg<sup>-1</sup> body weight served as the positive control. The rats were autopsied, worms counted, and the percentage activity calculated as described by Cavier, (1973), using the formula:



Where: N = Average number of worms found in control animals and n = average number of worms found in groups of treated animals. Anactivity of 50% was considered significant.

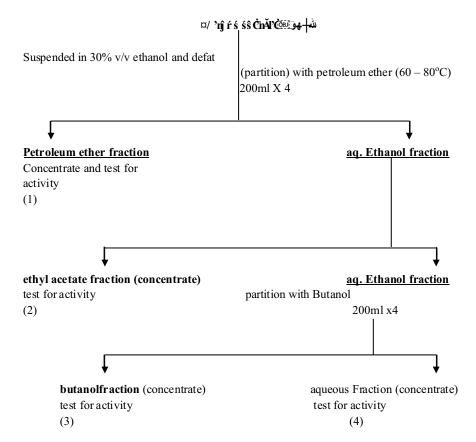


Fig. 1: Partitioning chart for crude extract of Annogeissus schimperi.

#### Statistical analysis

Data obtained from the experiment were subjected to statistical analysis for Analysis of Variance (ANOVA) one way test using Statistical Packages for the Social Science (SPSS) version 11. Results were expressed as mean±standard deviation. P value <0.05 were considered statistically significant.

#### RESULTS

The result of the phytochemical screening of the ethanol crude extract of *Anogeissus schimperi* revealed the presence of various constituents such as carbohydrates, alkaloids, tannins, saponnins and flavonoids as shown in Table I.

The results of the anthelmintic trials using the three fractions (AF, BF and EF) at50mg kg<sup>-1</sup> each of the extract of *Anogeissus schimperi* are represented in Table II. The result indicated that the aqueous fraction caused a highly significant

(P <0.05) activity of 62.22%, the ethylacetatefraction a significant (P <0.05) activity of 53.33% and the butanolfraction a non-significant (P > 0.05) activity of 28.88%. The result therefore indicated that the aqueous fraction of the ethanol extract of *Anogeissus schimperi* is the most effective against adult *Nippostrongylus braziliensis* in rats.

Table III shows the mean worm count and percentage clearance following administration of the portioned fractions of Anogeissusschimperi in rat model. All the rats were given the same dose (50mg kg<sup>-1</sup>) of the aqueous, butanol and ethylacetatefractions respectively. The average worm counts were 18, 6.8, 12.8, 8.4 and 0 respectively for rats given aqueous, butanol, ethylacetatefractions and Albendazole and distilled water. There was a significant diference (P <0.05) in mean worm count obtained for the three fractions at the same dosage.

Group constituents	Test	Observation	Inference
Carbohydrate			
General test.	Molisch's test R	eddening formed +++	
Alkaloids Dragendorff	"s		
-	reagent o	range red precipitate ++	
	Mayer's reagent	dark-brown precipitate	++
	Wagner's reagent	1 I	++
<u>Tannins</u>			
General	FeCl <sub>3</sub> test	very deep bluish-black	++++
colouration		•	
Saponins	frothing test	frothing observed	++
Flavonoids colour formation	FeCl <sub>3</sub> test	there was yellow	++

Dose Drug/extr	ract	A (mg l	verage (g <sup>-1</sup> )	_	0	recov Activit	•	
Control		0	18±3	.54		15-24		0.00
Albendazole	7.5		$0\pm0$	.0		0	10	00.00
Aqueous (A	F)	50	6.8=	±3.9		2 - 12	62	2.22
Butanol(BF)	50		12.8±2.	6	9 - 1	.6	28.88	3
Ethylacetate(EF)	50		8.4±6.	2	0 - 1	7	53.33	3

#### TABLE II: PERCENT ACTIVITY IN ANTHELMINTIC TRIALS USING THE THREE PARTITIONED FRACTIONS OF THE EXTRACT OF ANOGEIS SUSSCHIMPERI IN RATS GIVEN 200 L3 OF NIPPOSTRONGYLUS BRAZILIENSIS

TABLE III: MEAN WORM COUNT AND PERCENTAGE CLEARANCE FOLLOWING ADMINISTRATION OF THE PORTIONED FRACTIONS OF *ANOGEISSUS SCHIMPERI* IN RAT MODEL

<i>Treatment (</i> mgkg <sup>-1</sup> )	Mean Worm Count	Mean Percentage		
Control 0	$18.00^{\circ} \pm 3.54$	$0.00 \pm 1.77$		
Albendazole 7.5	$0^{\mathrm{a}}\pm0.00$	$100 \pm 0.00$		
Aqueous 50	$6.8^{a} \pm 3.90$	62.22±1.95		
Butanol 50	$12.8^{\rm c} \pm 2.59$	$28.8 \pm 1.30$		
Ethylacetate 50	$8.40^{b} \pm 6.19$	53.33±3.10		

<sup>a</sup>Highly Significant (P < 0.05)

<sup>b</sup>Significant (P < 0.05)

°Non-significant

Results expressed as  $\pm$ SD

#### DISCUSSION

Results of the phytochemical analysis of the crude extract of the bark of *Anogeissus schimperi* revealed the presence of tannins, Carbohydrate, Alkaloids, Sanponnins flavonoids among others. Arbonnier (2002) reported that the leaves, roots and bark of *Anogeissus schimperi* contained high levels of tannins and are used in different localities for tanning leather. The present study has also shown that tannins are present in the ethanol extract of the bark of *Anogeissus schimperi*.

Among the three fractions (Aqueous, butanol and ethylacetate) tested at 50 mg kg<sup>-1</sup> body weight, the aqueous and ethylacetatefractions gave a significant (P <0.05) activity when compared with the control, meaning that the anthelmintic ingredient in the bark of Anogeissus schimperi is distributed among these two fractions even though the aqueous fraction exhibited greater effect than the ethylacetatefraction.Thebutanolfraction showed the lowest activity of 28.88%. The fact that there was no significant difference (P>0.05) between the activity of the control and butanolfraction is indicative that this fraction did not contain Appreciable anthelmintic properties to exhibit good anthelmintic activity. It is evident from the results of this study that aqueous fraction of Anogeissus schimperi had higher anthelmintic activity compared to the ethylacetate and butanolfractions. This may be an indication of the presence of active anthelmintic principle(s) in the water - soluble fraction of the ethanol extract of Anogeissus schimperi.

The result obtained in this study justifies further investigation of the anthelmintic effect of the crude extract and particularly aqueous fraction of *Anogeissus schimperi* in higher animals. The result obtained in this study does not exclude the possibility that the less active butanolfraction of *Anogeissus schimperi do* possess anthelmintic property. This is because *Nippostrongylus braziliensis* is known to be more resistant to anthelmintics than most other strongyles (Standen, 1963; Cavier, 1973).

Among the three fractions, the aqueous fraction produced the highest activity of 64.15 % at 50 mg kg<sup>-1</sup>. This is the fraction that have, in addition to the tannin content higher concentration of flavonoids. The flavonoids together with the tannin, may have produced the high level of activity observed. Tannins are known to contain a mixture of phenols (Harbone, 1973) which are uncouplers of oxidative phosphorylation in helminth parasites. Phenols readily combine with plasma proteins rendering them resistant to proteolytic enzymes secreted by the worms (Mitcell *et al.*, 1983).

Flavonoids are believed to stimulate intestinal motility similar to that produced by acetylcholine (Akendenge, 1992) thereby causing rapid worm expulsion from the GIT. In this study, flavonoids were shown to be present in the bark of *Anogeissus schimperi*.

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