IN- VIVO EVALUATION FOR ANTHELMINTIC EFFECT OF ALKALOIDS EXTRACTED FROM THE STEM BARK OF *AFZELIA AFRICANA* IN RATS

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ABSTRACT

The anthelmintic activity of alkaloid extracted from the stem bark of *Afzelia africana* was evaluated *in-vivo* in rats experimentally infected with *Nippostrongylus braziliensis*. The alkaloids was obtained after partitioning the crude methanolic extract of the plant powdered material in petroleum ether, chloroform and N-butanol as solvent and subsequently subjecting them to phytochemical screening to identify the portions with the highest concentration of alkaloids which was then used in the study. The phytochemical screening shows that the amount of alkaloids was higher in chloroform and N-butanol portions. The anthelmintic activity was assessed by comparing the number of worms recovered from rats treated with the fractions to those from non-treated infected controls. This study considered deparasitization rate of 70% or greater as significant. The chloroform and N-butanol portions produced mean total worm count of 2.5 and 3.5 with a significant ($p<0.05$) deparasitization rates of 79.20% and 72.72% respectively when the maximum tolerated dose of 1000 mg/kg was administered. The deparasitization produced by the chloroform and N-butanol portions were significant ($p<0.05$) when compared to that produced by the placebo-treated negative control treated rats, while the deparasitization produced by the albendazole treated rats as a positive control was highly-significant ($p<0.001$). Thus this result needs further investigations to validate their efficacy in natural or experimental infection in ruminant.

Keywords: Alkaloid, *Afzelia africana,* Anthelmintic, Rat

1. INTRODUCTION

Herdsmen are generally very knowledgeable about the effectiveness of a lot of plants against common diseases and ailments affecting their livestock, like gastrointestinal parasitism in cattle. Even though Fulani pastoralist have a number of folklore remedies for a very long time (and they strongly believe in their efficacy), there were no scientific evidence to confirm such beliefs. Hence, studies are now being carried out not only to scientifically determine their efficacy [1-2], isolate the active principles; determine the mode of action [3-5] and their toxicity, but also to standardize the dosage [6]. The use of alternative drugs has been recommended as a measure to avoid the development of resistant strains of helminth parasites, reduce drug residue in animal products, and reduce environmental pollution and to explore the possibility of reducing cost of controlling helminthiasis [7-9].

Successes of use of herbal remedies in China, India and Russia have been recorded. For instance; a compound derived from herbs in China is now a drug of choice against cerebral malaria and benzolthiocyanate derived from the seed of C*arica papaya* were intensively used for the treatment of ascariasis in

children, dog and bird and oxyuriasis in mice [10]. Benzolthiocyanate was also found to be active against *Hymenolopis nana,* both *in-vitro* and *in-vivo* [11]. Tannins are secondary plant metabolites, which have been closely associated with plant defence mechanisms towards insects [12] and mammalian herbivores [13]. Tannins are usually divided according to their chemical structure and properties into two groups; hydrolysable and condensed tannins. The later is the most widespread group of tannins in nature and has been considered responsible for causing a number of detrimental effects towards monogastric [14] and ruminant herbivores [15]. However, ruminants can benefit from the presence of condensed tannins in their diets; the consumption of average concentration of condensed tannins can result in increase weight gain, wool growth, milk secretion [16] and decrease the detrimental effects of gastrointestinal parasitism [15]. Recent studies in ruminants suggest that parasitized sheep and red deer grazing on forages high in condensed tannins had lower faecal egg counts and worm burdens compared to those grazing on forages low in condensed tannins [17-18]. Recently, a direct anthelmintic effect of a plant extract high in condensed tannins (Quebracho) towards *T. colubriformis* population has been demonstrated [19]. Mangiferin is a major polyphenol in the

aqueous extract (vimang) acquired from *Mangifera indica*. In-vitro test showed that the polyphenol and the aqueous extract were effective against L2 and L3 stages of *Trichinella spiralis* in a mouse model. Moderate effects were reported against the L4 in the muscles but not against the adult parasites [20]. The *in-vitro* anthelmintic effects of embelin an extract from *Embelin schimperi*, was evaluated in mice/ rats infected with the cestodes *Hymenolepis microstoma* and *H. diminuta* and in mice infected with the trematodes *Echinostoma caproni*, and the nematode *Heligmosomoides polygyrus*. The extract had activity only against the cestodes [21]. Atanine, a quinolone alkaloid extracted from dried fruits of *Evodia rutaecarp*, inhibited *in-vitro*, the motility of free-living stages of the trematode *Schistosoma mansoni*, and also had activity against the nematodes *C. elegans* adult as well as the larval stages of *Teladorsargia circumcinta* [22]. B- sitosterol (from leaves of *Mentha cordifolia*), has been tested in an *in vitro* assay against the adult of the porcine roundworm *Ascaris suum*, and found to have similar activity to mebendazole a synthetic anthelmintic [23]. Flavan-3-ols (the monomer units of condensed tannin), and their galloyl derivatives were evaluated *in vitro* on the viability of eggs, the development and the viability of the free-living stages of *Trichostrongylus colubriformi;* and was found to inhibit egg hatch in addition to impairing the development of larvae [24].

Afzelia africana SM is a wide spread tree species mostly found in savanna fringing forest and drier parts of forest regions and commonly referred to as mahogany, kawo, apa and akpalata in English, Hausa, Yoruba and Igbo respectively [25]. The seed is widely used for medicinal purposes, for industrial use as in soap, margarine, and candle making and as diets such as condiments and thickening in soup [26]. Atawodi [27] identified the use of herbal preparations involving *Afzelia africana* in the treatment of helminthiasis.

2. MATERIAL AND METHODS

The stem barks of *Afzelia africana* '*SM*' [25] were collected, sun-dried and pulverized into powdered form using mortar and pestle and sieved as described by Onyeyili [28]. The powdered plant material was extracted using 2.5litres of absolute methanol in a soxhlet apparatus (Quick fit corning Ltd. A division of Stafford England); after which the solution was evaporated to dryness in a vacuum using a rotary evaporator coupled to a thermo-regulator.

The crude methanol extract of the plants was suspended in 200 ml of 30% v/v aqueous methanol. This was then partitioned in a step-wise manner in 150 ml of petroleum ether, chloroform and N-butanol each as solvent using separating funnel. After evaporating the solvents, the portions were subsequently referred to as petroleum ether, chloroform and N-butanol portion and were tested for anthelmintic activity on rats infected with *Nippostrongylus braziliensis* [4-29].

The extracts were subjected to phytochemical tests using standard techniques described by Trease [30] and Brain et al [31]; for the presence of alkaloids. The portions that had the highest concentration of alkaloids were chosen for the anthelmintic evaluation.

Evaluation of maximum tolerated dose (MTD) of the crude methanol extract

Due to lack of information on the precise dosage of the plants preparations as used by the traditional herdsmen and pastoralists, a maximum tolerated dose (MTD) experiment as described by Loomis [32] and Lorke [33] was carried out on the crude methanolic extract. The established MTD was then used as the basis for the administration of the plant extract in the anthelmintic activity studies.

2.1. Experimental infection/Design

Each of the thirty (30) worm-free rats were infected subcutaneously in the cervical region with 200 viable L3 of *N braziliensis* in 0.2 ml of water using an 18-gauge needle attached to an insulin syringe [29]. Five days post infection; fresh faecal samples from each infected rat were collected by squeezing it out of the rectum; and examined quantitatively for *N braziliensis* egg using the simple floatation method [34]. Rats not shedding ova of *N braziliensis* were excluded from the experiment.

The infected rats were randomly allocated to three (3) experimental groups (A-C) for each of the plant extract. Group A (positive control group) having six rats, were treated with albendazole at 200 mg/kg body weight [29], group B sub-divided into two groups of six rats each for evaluation of the chloroform and N-butanol (i.e. the portions that contains alkaloids) based on the MTD [3]; whereas group C (negative control) were subdivided into two groups of six rats each and given water and propylene glycol as placebo based on the maximum convenient volume (MCV) of 5 ml/kg [4].

2.2. Treatment with extracts

Oral treatment with the extracts was carried out on day seven (7) post infection. Before the treatment all rats were weighed to determine the appropriate dose and the maximum convenient volume (MCV) for individual rats. Observation was made daily for three days for abnormal behavioral signs as a result of ingesting the extracts [35].

2.3. Worm counts

On Day 2-post treatment, all the rats were fasted for 24 hrs, and salvaged for adult worm count using the WAAVP guides [36]. The first 15 cm of the small intestine was removed, cut longitudinally and placed between two clean 20

cm glass slides. The section was examined at x40 magnification of a dissecting microscope. Visible worms were counted and recorded [29]. The extract that caused the highest reduction in worm count without producing any behavioral changes in the rats was considered to be the most active fraction.

2.4 Percentage efficacy (Deparasitization)

Percentage efficacy (deparasitization) of the various fractions of each plant was calculated according to the method of [37]:

$$\% \text{ Efficacy} = \frac{N-n}{N} \times 100\%$$

N= number of worms counted in the placebo-treated rats
n= number of worms counted in the plant extracts or albendazole-treated rats.

It was considered 'a priori' that the efficacy of the plant extracts would be biologically signifacant if a reduction in total worm count (TWC) above 70% occurred [35].

2.5. Statistical Analysis

Means of data obtained were analyzed statistically using the software package for GraphPad prism (version 4.0-----2003). Statistical significance for the anthelmintic effect of crude methanol extract, chloroform, N-butanol portion and aqueous portion was assessed by ANOVA; subsequently Borferroni's multiple comparison tests. P value < 0.05 was considered significant.

3. RESULTS

Following subjecthon of 500 g of plant material to extraction with 2.5 liters of absolute methanol, the plant yielded 80 g (16%) as crude methalonic extract. Petroleum ether, chloroform and N-butanol used as separating solvents yields 3 g (15%), 7 g (35%) respectively; leaving 10 g (50%) as the aqueous methanol fraction. following fractionation of 20 g crude methalonic extract

3.1. Phytochemical screening

The aqueous methanol, chloroform and N-butanol fractions were seen to have more of alkaloids and only a trace was in crude methanol extract portions while it was absent in petroleum ether.

3.2. Determination of maximum tolerated dose (MTD)

At a dose range of 10 to 1000 mg/kg, *Afzelia africana* did not cause any visible toxic effect on the rats as they were active 6 hrs after recovering from the stress due to administration procedure. On the other hand, from the dose range of 1600 to 5000 mg/kg, the rats demonstrated varying degrees of signs of toxicity which manifested as visible body weakness, inability to move and reduced appetite from 24 hrs to 7 days post administration (Table 2). Therefore the doses of 1600 to 5000 mg/kg were considered unsafe for the rats; and the dose of 1000 mg/kg body weight was chosen as the experimental treatment dose for both the crude methanol extracts and the various fractions.

Table 1: *Phytochemical screening for the CME and various portions of A. Africana*

| Test | Alkaloid content in | | | | |
	Crude methanol	Pet ether	Chloroform	N-butanol	Aqeous methanol
Draendroff's	-	++	+++	+	-
Mayer's	+	+	+	++	-
Wagner's	+	+	+	+	-

Table 2: Maximum tolerated dose/toxicity of crude methanol extract of Afzelia Africana

Dose(mg/kg)	10	100	1000	1600	2900	5000
Initial number of rat	3	3	3	3	3	3
Mortality	0	0	0	0	0	0
Observation	a	a	a	b	c	d
Inference	-	-	-	+	++	+++

Key: *a = rats active 6-24 hrs and beyond; b = rats showed weakness for more than 24 hrs; c = rats showed weakness for more than 48 hrs; d = rats showed weakness for more than 7 days; - = no sign of toxicity; + = slightly toxic; ++ = toxic; +++ = more toxic*

3.3. Anthelmintic effect of extracts on *N. braziliensis*

Rats that had oral infection of 200 L3 followed by treatment with chloroform (1000 mg/kg), N-butanol (1000 mg/kg) fractions and albendazole (200 mg/kg) had respective mean worm count of 2.5, 3.5 and 0; compared to the mean worm count (12.83 and or 12.0) from the negative controls rats. The extract fractions produced percentage deparasitization of 79.20% (chloroform) and 72.72% (N-butanol). Albendazole (positive control), gave 100% deparasitization compared to the crude methanol extract and the respective fractions whereas the negative controls gave 0% deparasitization.

The deparasitization produced by the chloroform and N-butanol fractions were significant (p<0.05) when compared to that produced by the placebo-treated negative control rats, while the deparasitization produced by the albendazole extract was highly-significant (p<0.001).

Table 3: Worm count and percentage deparasitization 7 days after treatment with crude methanol extract and fractions of A. africana

Rat	Worm count after treatment with				
	Chloroform (1000mg/kg)	N-butanol (1000 mg/kg)	Albendazole (200 mg/kg)	Placebo1(5 ml-water)	Placebo2 (5 ml-p. glycol)
1	2	4	0	4	6
2	3	3	0	17	16
3	4	2	0	18	15
4	1	5	0	12	10
5	3	1	0	14	11
6	2	6	0	12	14
Mean±SD	2.5 ± 1.05a*	3.5 ± 1.87a*b	0.0 ± 0.0a*	12.83± 5.0	12.0 ± 3.74
%DPZ	79.20	72.72	100	0	0

Mean with * within the column are significantly different at p<0.001, while those with the letter "a" and "b" show no significant difference between their means at p>0.05 as determined by Borferroni's multiple comparison test; %DPZ= percentage deparasitization

4. DISCUSSION

Different methods exist for the extraction and separation of plant materials for pharmacological and medicinal uses. In this study, exhaustive extraction of the dried powdered material of plant bark with a polar solvent (methanol) and the stepwise partitioning in petroleum ether, chloroform and N-butanol was used.

Recent harmonizations on anthelmintic efficacy guidelines in ruminants have indicated that for a drug to be considered efficacious, a 90% reduction in total worm count (TWC) should be achieved [3]. However, the *in-vivo* anthelmintic effect of alkaloids extracted from *A. africana* is unknown. Thus, it was considered 'a priori' that the efficacy of the alkaloid extract of the plant would be biologically significant if a reduction in total worm count above 70% occurred [35]. Treatment of rats with alkaloids rich solvent-partitioned extracts (i.e. chloroform and N- butanol,) of the plant showed anthelmintic activity. The chloroform fraction of the extract produces a total reduction of worm count of 79.20%. Also the N-butanol had the required significant biological reduction in total worm count of 72.20% compared to the untreated control groups. The result of this study equally demonstrated that the parasite *N. braziliensis* was highly sensitive to albendazole (positive control) with complete deparasitzation at a dose rate of 200 mg/kg body weight [29].

Partitioning plants extracts is a method of separating plant components based on their solubility in the solvents used [38]. The N-butanol fraction was soluble in water and other polar solvents like alcohol, suggesting that the constituent of this fraction are mainly polar compounds. However, the chloroform fraction was only soluble in propylene glycol (a non polar solvent). This informed the inclusion of propylene glycol as one of the placebo.

The outcome of the phytochemical screening revealed the plants is rich in alkaloids. Alkaloids are nitrogenous compounds which occur in plants. Many are optically active and they are basic in nature and form salts with plant acids [31]. Their solubility show considerable variation and depend upon whether they are present as the salt or as the free base. Their salts are soluble in water and insoluble in organic solvents [31]. In *in-vitro* and *in-vivo* studies [39] reported that alkaloids extracted from both the latex and leaves of C*alotropis procera*, was effective in inhibiting the exsheatment of L3 of *H. contortus* to L4 in sheep. Lateef et al [40] also reported that alkaloids and their glycosides extracted from the root of *Adhatoda vestica* was effective against mixed gastrointestinal infections in sheep. Also, Onyeyili et al [28] reported that tannins and alkaloids, the active principles of *Nauclea latifolia* bark, were effective against mixed infections in sheep. The present study has shown that alkaloids are present in chloroform and N-butanol, with only traces in the crude methanol extract and absent in the aqueous methanol fraction. It is possible that the presence of alkaloids have had a significant deparasitization observed.

5. CONCLUSION

Result from this study demonstrated that the chloroform and N-butanol fractions of *A. africana* contain alkaloid that is effective against experimental *N. braziliensis* infection in rats at a non-toxic dose of 1000 mg/kg; whereas albendazole was found to be highly efficacious. However, the efficacy of alkaloids is comparable to that of albendazole (a conventional anthelmintic) at a dose rate of 200 mg/kg. The *in-vivo* model was also found to be a useful tool for rapid screening of

anthelmintic activity of plant preparations against nematode parasites. The investigation of chemical compounds from natural products is fundamentally important for the development of new anthelmintic drugs, especially in view of the vast worldwide flora. Thus a quality controlled extraction of *A. africana* and the isolation of the bioactive compounds could be a promising alternative to conventional anthelmintic for the treatment of gastrointestinal helminths of ruminant in the future. Such a treatment could be used in control strategies against gastrointestinal nematodes in organic and conventional production systems.

More detailed studies are needed to isolate, characterize and evaluate the active components and the mechanism of action of the identified active principles. Also, studies on the toxicity, evaluation of the effect *in-vivo* against economically important gastrointestinal nematode species and the establishment of adequate doses for sheep, goats and cattle are needed.

6.   REFERENCES

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