Efficacy of *Tephrosia vogelli* and *Vernonia amygdalina* as anthelmintics against *Ascaridia galli* in indigenous chicken

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Abstract

The efficacy of *Tephrosia vogelli* and *Vernonia amygdalina* leaf extracts as anthelmintic against *Ascaridia galli* was determined *in-vitro* and *in-vivo*.

The extracts whose chemical constituents included rotenoids, sesquiterpene lactones, glycosides, anthracenes and tannins, had significant ($p<0.05$) activity against *Ascaridia galli* both *in-vitro* and *in-vivo*. The *in-vitro* larval migration inhibition of 74.7 and 63.7 % was supported by the faecal egg count reduction of 77.4 and 76.9 and reduced total worm counts at necropsy for *Tephrosia vogelli* and *Vernonia amygdalina* extracts, respectively.

The results have demonstrated that the extracts of the two plants have significant activity against the chicken parasite *Ascaridia galli* and can be integrated in indigenous chicken health management system.

Key words: anthelmintic, Ascaridia galli, efficacy, indigenous chicken, Tephrosia vogelli, Vernonia amygdalina

Introduction

Helminth infections in indigenous chicken are very common because of the risks posed by the production system which predominantly relies on scavenging (Ondwassy et al 2000) and contributes to the low production of the indigenous chicken (Siamba et al 2000, Sani et al 1987). Control of the parasites in rural poultry using commercial anthelmintics in Kenya is rare (Siamba et al 2000) largely because of high relative costs. Instead, the farmers use extracts from indigenous plants for treatment and management of helminthosis and other diseases. Despite their widespread use, few studies have been carried out in Kenya to verify the effectiveness of plant extracts used as anthelmintics especially in chicken. This paper presents results of a study designed to validate two indigenous plants as anthelmintics against the chicken parasite *Ascaridia galli*.

Materials and methods

Plant collection and identification

Plant specimens including the stalk, leaves, flowers and pods, were collected from the field and submitted to the National Museums of Kenya-Herbarium for identification. The plants were identified as *Tephrosia vogelli* and *Vernonia amygdalina* belonging to the Fabaceae and Compositae families, respectively.

Preparation of the plant extracts

Traditionally, about 1 kg fresh leaves from the two plants are pounded and made into a suspension with 1 litre of warm water. The suspension is left standing for 8 hours with occasional shaking. The suspension is squeezed through a double-layered piece of cloth to recover the juice. This stock extract is either diluted and offered in drinking water for at least 5 days or severely affected birds are directly drenched. In this experiment, 1 kg of fresh leaves of *Tephrosia vogelli* and *Vernonia amygdalina* were chopped into small pieces and blended into a uniform suspension in 1 litre of warm distilled water. The suspension of each extract was soxhalated for 8 hours. After cooling, the contents were filtered successfully through ordinary then through Whatman filter paper No 1. The filtrate and the residues were collected separately. The filtrates of respective plants were concentrated in rotatory evaporator connected to a vacuum pump to dryness. The yields were weighed and stored at -20oC until used. The extracts were subjected to various chemical analysis for constituents including retenoids, sesquiterpene lactones, tannins, anthracenes and glycosides by standard methods (Harborn 1976, Ioan 1982).

Parasites

The method used to obtain *Ascaridia galli* larvae for *in-vitro* larval migration inhibition test was a modification of that described by Hurwitz et al (1973). Briefly, adult *Ascaridia galli* parasites were obtained from the intestines of indigenous birds found infected with *Ascaridia galli* by screening for eggs in faeces. The parasites were washed with distilled water and dilute formaline solution to avoid contamination. Their uteri were squeezed out and the ova liberated by manipulation of the uteri. The ova were washed several times with phosphate buffered saline (BPS), sedimented by centrifugation and finely dispersed in 2% hydrated copper sulphate ($CuSO_4$ $7H_2O$) solution. They were kept at room temperature for 4 weeks before use. Fertile ova, identified by the appearance of the larvae, were counted using a haemocytometer.

The ova, prepared as described above, were diluted to obtain the required dosage of a bout 5,000 larvated ova of *Ascaridia galli*. Three 5-weeks old indigenous birds were infected by crop intubation. They were then maintained on battery cages for 4 days. They were slaughtered and the larvae recovered from the lumen of the intestines. The intestinal contents were centrifuged at 1000 g and sediment repeatedly washed with phosphate buffered saline (PBS). The number and viability (judged by their motility) of the larvae estimated microscopically.

In-vitro screening of plants

Laboratory evaluation of anthelmintic activities of the selected plants was done using *Ascaridia galli* larvae recovered as described above. The *in vitro* larval migration assay described by Wagland et al (1992) was adapted with modifications. Briefly, about 100 active larvae in 750 µl of larval suspension were pre-incubated in neat (dried extracts reconstituted to original volume in distilled water) plant extracts at room temperature (about 23^{o}C) for 4 hours and then transferred to larval migration inhibition (LMI) tube using Pasteur pipette. The bottom of LMI tube was made of nylon mesh with a pore size of 30µm. The LMI tube was then placed in a 48-well tissue culture plate (Coster, Cambridge, MA, USA) ensuring that the bottom of the LMI tube remained above the bottom of the well. Positive and negative control tubes containing 100 larvae in 0.35 mg/ml water-soluble Piperazine citrate (Ascarex®, Cosmos, Nairobi, Kenya) and PBS, respectively, were also set up. The set up for each plant extract and controls was performed in quartriplates. The entire set up was allowed to stand at room temperature for 16 hours after which the *Ascaridia galli* larvae which had migrated into the wells were counted. Subsequently, the relative larval migration percent inhibition (PI) was estimated using the following formula adopted from the World Association for Advancement of Veterinary Parasitology (WAAVP) (Coles et al 1992):

$$PI = 100\ (1 - XT/XC)$$

Where XT and XC are the arithmetic mean of the replicates for the treated and the untreated controls, respectively

In-vivo assays

Twenty eight (28) indigenous cockerels were artificially infected with larveted A*scaridia* eggs and patency monitored by faecal egg counts (MAFF 1986) . Following patency, the birds were divided into 4 groups of 7 birds each. Group 1, 2, and 3 (designated as T_1, T_2 and T_3, respectively) were dosed with commercial anthelmintic (Piperazine citrate: Ascarex®, Cosmos, Nairobi, Kenya), *Tephrosia* and *Vernonia* extracts,

respectively. The fourth group T_0 was left as untreated control. Faecal samples for Faecal egg counts were collected from each bird on day 0 (pre-treatment epg) and day 14 (post-treatment epg). After 14 days post infection, the birds in all the groups were necropsied and parasites isolated and quantified as described by Wilson et al (1994). Faecal egg count reduction test (FECRT) and total worm counts (TWC) reduction were used to estimate the efficacy of the plant extracts. The efficacy by FECRT was calculated on the basis of the differences in egg counts between treated and control groups and was corrected for the changes that occurred in the control group by the following equation:

$$(FECRT)\% = [1-(T_2/T_1 \times C_1/C_2)] \times 100$$

where T and C are the geometric epg means for the treated and control groups and subscript 1 and 2 designate the counts before and after treatment, respectively (Campbel et al 1978).

Statistical analysis

The percent efficacy between treated and control groups and between treated groups was compared by independent t-test (Sokal and Rohlf 1981). The means of pre- and post treatment epg and the number of adult worms recovered at necropsy were analysed by F-test.

Results

The extracts of *Tephrosia* and *Vernonia* gave a yield of 14.3 and 11.8% w/w respectively. They were both dark red in colour and odourless. Some chemical constituents of the extracts are shown in table 1.

Table 1. Chemical constituents of extracts of test plants

Yield/Chemical constituents	Plant extract	
	Tephrosia	Vernonia
% weight/weight	14.3	11.8
Rotenoids	+	-
Sesquiterpene lactones	-	+
Glycosides	+	+
Anthracenes	+	-
Tannins	+	+

(+) - Present; (-) Undetectable

In-vitro assay of the extracts indicated significant differences ($P< 0.05$) between mean larval counts in plant extracts and commercial anthelmintic treated groups and the control (Table 2). Thus 74.7, 63.9 and 100% of the larvae were inhibited by *Tephrosia*, *Vernonia* and Piperazine citrate, respectively.

Table 2. Mean numbers and relative percent inhibition

	Extract/drug			
	Tephrosia	Vernonia	Ascarex®	Control
Mean No. of larvae in well	21.4 ± 3.2	30.6±8.1	0	84.7±11.4
Relative % inhibition	74.7	63.9	100	-

Table 3 shows the mean epg and total worm counts (TWC) of experimental birds. The epg before treatment showed no significant difference ($p<0.05$) between the control group and the treated groups (T_1, T_2, T_3).

Table 3. Mean number of eggs per gram of faeces (epg) and total worm counts (TWC) at necropsy.

Treatment	Mean epg				Mean TWC
	N	Before treatment	After treatment	Efficacy, %	
T_1	7	1050 ^{a}	0 ^{a}	100 ^{a}	0 ^{a}
T_2	7	850 ^{a}	237.5 ^{b}	77.4 ^{b}	3.0 ^{b}
T_3	7	1112 ^{a}	325 ^{b}	76.9 ^{b}	3.25 ^{b}
T_0	7	845 ^{a}	1070 ^{c}		21.6 ^{c}

$^{a\ bc}$ figures with same superscript in the same column do not differ significantly ($p<0.05$)

However, after treatment, the epg of T_1, T_2, and T_3 was significantly ($p<0.05$) reduced compared to the control (T_0). Comparisons of epg of the treated groups showed that there was no significant difference between T_2 and T_3 but both had significantly higher epg counts compared to T_1 at 5% level of confidence. The worm burden was significantly lower in T_1, T_2, T_3 compared to the controls (T_0).

Discussions

Plants are known to synthesis many chemical compounds that posses as many biological activities (Klocke 1989). Some of the compounds exhibit specific and unique properties that have long been exploited in animal and human medical systems. By influencing metabolic pathways, natural plant compounds remain the major source of medicines for management of animal and human health. Phytochemical results in the present study indicate that the two plants contain various chemical compounds including retonoids, sesquiterpene lactones, glycosides, anthracenes and tannins. Some of these compounds especially sesquiterpene lactones and rotenoids (Bizimana and Scherecke 1996, Robinson 1975, Klocke 1989) have been reported elsewhere to have biological activities against helminth.

Results obtained in this study indicated that single doses of *Tephrosia vogelli*, and *Vernonia* leave extracts have significant *in-vitro* and *in-vivo* anthelmitic activity against *Ascaridia galli*. However, under normal farming circumstances, the birds are dosed in drinking water for a few successive days. This practice might have more beneficial effects of the extracts and is worth being investigated. The results also demonstrated that the plant extracts not only depress faecal egg output but also significantly reduce the adult worms population in parasitised birds. This is desirable as it has the advantage of reducing the deleterious effects on individual birds and contamination of the environment with parasite eggs.

The actual active component (s) of the extracts remains unknown so is the interaction of the chemical components and their effects on the parasites. More studies are therefore needed to determine the active principles for further pharmacological and toxicological assessment. However, the results obtained in this study have shown that there exists potential parasite control system in rural poultry based on indigenous plants and provides an opportunity to integrate this plants in health care system of indigenous poultry.

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