Original Research Article

Anticoccidial effects of *Ageratum conyzoides* (Asteraceae) and *Vernonia amygdalina* (Asteraceae) leaves extracts on broiler chickens

Abstract

Avian coccidiosis is a parasitic disease which causes considerable economic loss in poultry. The emergence of anticoccidial drug resistance enhances the need for development of novel approach and alternative controls strategies such as plants extracts. Therefore, this study was conducted to evaluate the anticoccidial efficacy of ethanolic extracts of *Ageratum conyzoides* and *Vernonia amygdalina* on broiler chickens. Ninety (90) Cobb 500 broiler chickens were divided into nine groups of 10 chickens each. Each chicken in 8 groups (A-H) was orally infected with approximately 3000 sporulated oocysts of *E. tenella* at day 28 of age while one group (group I) served as uninfected control. After establishment of the disease at day 7 post-infection, chicks of groups A to F were treated with the graded concentrations (1.5, 3 and 6 g/ l) of ethanolic extracts of both plants. Group G was treated with the conventional drug (Anticox) and group H served as infected non treated control. All treatments were mixed with drinking water and administered for five consecutive days. The activity was evaluated by means of fecal oocyst count reduction, host growth and haematological parameters. The results showed that, ethanolic extracts of both plants demonstrated a gradual inhibitory effect on the shedding of oocysts in a concentration-dependent manner. Among the treated groups, the highest inhibitory effect was recorded in the extract concentration of 6 g/l (oocyst count reduction rate of 100% which was comparable to the group receiving conventional drug (P>0,05)).There were no significant differences in the food intake between experimental groups (P>0,05). The mean body weight of treated groups was significantly (P<0,05) higher than that of the untreated group. All treated groups showed better feed conversion ratio (FCR) as compared to infected non-treated group (P<0,05). The mean of RBC count, Hb rate and PCV after treatment with the various concentrations of ethanolic extracts of both plants was significantly (P<0,05) higher than those of the untreated group. These results demonstrated that both plants have similar activity and could therefore find application in anticcocidial therapy.

Keyword: Anticoccidial activity, *Ageratum conyzoides*, *Vernonia amygdalina*, *Eimeria tenella,* coccidiosis.

Introduction

Poultry plays an important role for mankind through food supply, income and employment generation, providing raw materials to some industries. However, high mortality rates due to coccidiosis constitutes one of the greatest constraints on chicken development (Nghonjuyi *et al*., 2015). The disease has a significant economic impact on the poultry industry especially in broiler chickens due to its association with impaired growth, poor feed utilization, impaired feed conversion and poor weight gain leading to poor performance of chicken and hence mortality (El Banna *et al*., 2016; Abbas *et al*., 2017). Seven species are known to infect poultry and each species have its own characteristics depending on the site of infection and pathogenicity (Abbas *et al.,* 2015). Among these species, *E. tenella*, which causes caecal coccidiosis is the most pathogenic and causes heavy economic losses to commercial poultry farming (Alzahrani *et al*., 2016).

In Cameroon and other developing tropical countries, coccidiosis is controlled using anticoccidial drugs which are administered in feed or water. Success has been achieved by using these drugs but, the main problem associated with their poor response is the development of resistance in *Eimeria* species to the commonly available anticoccidial drugs and thus, these drugs are becoming less effective (Blake Damas *et al*., 2014). Along with this problem of drug resistance, there are also food safety and public health concerns about drug residues in poultry products (McDougald *et al*., 1998) which limit their use. According to Yang *et al.* (2015), anticoccidial vaccines are an alternative means to prevent coccidiosis. However, efficacy, safety and cost effectiveness are still challenging for these vaccines use in poultry (Sharman *et al*., 2010). Therefore, there is an expedient need for an alternative approach to control avian coccidiosis. The investigation of natural product as anticoccidial drugs holds promise as an alternative in the control of avian coccidiosis. Many studies have reported the *in vivo* efficiency of natural plant extract in the treatment of coccidiosis (El-Khtam *et al*., 2014; Gotep *et al*., 2016; El Banna *et al*., 2016; Abbas *et al*., 2017 and Wang *et al*., 2018).

Ageratum conyzoides and *Vernonia amygdalina* are two plants belonging to the Asteraceae family commonly known as 'King of herbs' and 'Bitter leaf' respectively. They are used in Mbouda-Cameroon to treat intestinal protozoan and gastrointestinal tract related complications. Literature reviewed revealed that, they possess many pharmacological properties such as antioxidant, antimicrobial, antiprotozoal, anthelmintic, and anti-inflammatory (Masengo *et al.,* 2015, Yeap *et al*., 2010). The leaves of these plants contain many bioactive compounds which are responsible for its diverse biological activities.

Looking at these medicinal properties, the current study was conducted to evaluate the anticoccidial activity of ethanolic extracts of *A. conyzoides* and *V. amygdalina* on broiler chickens experimentally infected with *E. tenella* oocysts.

2- Materials and methods

2-1 Plant collection and storage

The leaves of *A. conyzoides* and *V. amygdalina* were collected in the Bamboutos Division, Western Region of Cameroon and identified by the Cameroon National Herbarium (Yaoundé) using a voucher specimen registered under the Reference N° 6575 /SRF and N°

9535/SRF for *A. conyzoides* and *V. amygdalina* respectively. The collected plant material was dried in shade, at ambient temperature for about two weeks after which it was blended into fine powder and stored in airtight plastic bags in the laboratory at 4°C.

2-2 Preparation of the extracts

Cold extraction was done with 95 % ethanol for 72 h at room temperature. The mixture was daily stirred to permit better extraction of the active ingredients. The solution was sieved and filtered through a cotton layer and a filter paper of pore size 2.5 µm. The filtrate was evaporated in a rota vapor at 82ºC for 8 hours. The extract obtained was then poured in a large Petri dish and allowed to dry at room temperature for two days (Wabo Poné *et al*., 2006). Ethanolic extracts obtained were kept in a refrigerator at 4ºC for further processing.

2-3 Source of oocysts

Coccidials oocysts of *E. tenella* were obtained from the caeca of naturally infected chicks from a local market of Dschang Menoua Division, Western Region of Cameroon. Following evisceration at post mortem, the caeca were separated, sliced open longitudinally and their contents washed into a beaker using tap water. The washings were put in tube for centrifugation. Oocysts mensuration was done to determine the purity of the oocysts suspension obtained (Conway and McKenzie, 2007). Harvested oocysts were inoculated in three healthy chicks which served as reservoir chickens for coccidian oocysts. The chick was routinely monitored for the development of clinical signs of coccidiosis and the presence of *E. tenella* oocysts in their faeces (Adamu mesherem *et al*., 2013; Hassan Habibi *et al*., 2014). Ten (10) days post infection, fecal materials were collected and the oocysts were separated by sieving and sedimentation techniques. The collected oocysts were allowed to sporulate at room temperature in 2.5% potassium dichromate solution. Sporulated oocysts were cleared, counted and diluted to a final concentration of 3000 oocysts/ml of the solution using the McMaster technique as described by Messai (2015).

2-4 Birds and management

After leaving the hatchery, a total of 100 one day old Cobb broilers chickens of both sexes were grown under uniform brooder conditions from a one day old to experimental age. The birds were housed as a single group in a disinfected deep litter system with wood shavings as bedding material. The broilers chicks were reared under standard management practices in the animal house of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang (FASA). The litter was stirred three times a week till day 21 to prevent cake formation. Litter material when found damp was replaced by new one. All chicks were offered broiler starter ration for first three weeks followed by broilers finisher ration till the end of the experiment. Feed and water were provided *ad libitum*. Chicks were vaccinated for Newcastle Disease, Infectious bronchitis and Gumboro disease according to the programs schedule benched and applied in the F.A.R (Ferme d'Application et de Recherche) of the University of Dschang. At 22 days of age, birds were transferred in a suspended wire meshed (battery system) cages and acclimated till 28 days of age.

2-5 Evaluation of anticoccidial activity

Chicks were grouped into six (6) experimental groups A, B, C, D, E and F having 10 chicks each with 5 replicate by random allocation. Underweight and weak chicks were excluded from the experiment. All groups except group I (uninfected control) were orally infected with 3000 *E. tenella* sporulated oocysts. Daily collection and screening of faeces were carried out to check for oocyst presence. At day 35 (day 7 post-infection) after establishment of the infection they were treated with ethanolic extracts of *A. conyzoides* and *V. amygdalina* as well as the recommended drugs according to the followings schedule.

Group A: infected and treated with the extract of *A. conyzoides* at 1, 5 g/l.

Group B: infected and treated with the extract of *A. conyzoides* at 3 g/l.

Group C: infected and treated with the extract of *A. conyzoides* at 6 g/l

Group D: infected and treated with the extract of *V. amygdalina* at 1, 5 g/l.

Group E: infected and treated with the extract of *V. amygdalina* at 3 g/l.

Group F: infected and treated with the extract of *V. amygdalina* at 6 g/l

Group G: infected and treated with Anticox.

Group H: infected and received 0, 2 % Tween 80 (infected non treated control)

Group I: Non infected- non treated.

All treatments were mixed with drinking water and administered for five consecutive days.

2-6 Evaluation of the tested product efficacy

2-6-1 Mean oocysts count and oocysts reduction rate

Fresh faecal samples were collected from each replicate in all the groups on day 7 post infection (day 35 of age) and subsequently at three days intervals until the end of the study. The modified McMaster technique as described by (Thienpont *et al*. (1979) was used to estimate the oocysts per gram (OPG). The fecal oocyst concentration reduction rate was determined using the formula of Nghonjuyi *et al.* (2015) below:

$$\text{Fecal oocyst concentration reduction rate }(\%) = \frac{\text{Initial mean OPG} - \text{Final ean OPG}}{\text{Initial mean OPG}} \times 100$$

2-6-2 Growth performance

Performance of broilers was evaluated by recording the weekly body weight (BW) and daily feed intake. These parameters were used to calculate the feed conversion ratio (FCR) using the formula below as demonstrated by Abbas *et al.* (2017):

$$\text{FRC} = \frac{\text{Mean feed consumed}}{\text{Mean weight gain}}$$

Quantification of feed intake was done daily by making the difference between the weight of initial food and that of remaining food. Body weight of chicks were recorded at 7th day and then weekly for each treatment. In each week, birds were weighed early morning prior to feeding.

2-6-3 Haematological parameters

At the end of the experiment, three chickens from each replicate group were randomly selected and sacrificed, blood samples were collected from their aortic veins into a well labeled sterilized EDTA tube for haematological analysis.

2-7 Statistical Analysis

The data obtained from the study was summarized as mean ± standard error of means. Statistical comparisons between the treatment groups were made by one- way analysis of variance. Means were considered significant at $P<0,05$ and the means separated using Duncan's multiple range test.

3- Results

3-1 Faecal oocysts count and oocysts reduction rate

Ethanolic extracts of *A. conyzoides* and *V. amygdalina* demonstrated a gradual inhibitory effect on the shedding of oocyst in faeces during day 1-12 post- treatment in a concentration-dependant manner **(Table 1).** In the infected-non treated group, oocyst numbers rose rapidly to attain peak count 10 days post-infection after which they reduced progressively throughout the duration of the study. While in the uninfected untreated group it remained zero till the end of the study. Among the ethanolic extracts treated group, the highest inhibitory effect on oocyst shed in faeces was recorded in the group treated with 6 g/l of both plants with oocyst count reduction rate of 100% which was comparable to the group receiving standard- anticoccidial drug ($P>0,05$). In the group treated with 3g/l, the oocyst reduction rate was of 99,05 % and 98,02 % (for *A. conyzoides* and *V. amygdalina* respectively) while it was 93,54% and 96,12% (for *A. conyzoides* and *V. amygdalina* respectively) at 1, 5 g/l. The differences in oocyst reduction rates between the treated groups were not significant (P > 0.05) except with the untreated group (P < 0.05).

3-2 Effects ethanolic extracts of *A. conyzoides and V. amygdalina* on host growth parameters

The growth parameters of *E. tenella*- infected chickens treated with ethanolic extract of *A. conyzoides* and *V. amygdalina* are presented in **Table 2**. There was no significant difference (P>0,05) in feed intake between treated and control group. Also, there were significant differences (P<0,05) between different groups in mean body weight gains. Chickens in the non-infected-non treated group (NINT) had the highest mean body weight while chicken in the infected non treated (INT) group had the lowest mean body weight. The mean body weight gain of ethanolic extracts treated groups and the Anticox treated group (ITA) were significantly higher (P<0,05) than that of the infected non treated (INT) group. Among the ethanolic extracts treated groups, the highest weight gain was recorded by the group treated with 6 g/l (1008,90±111,90^{ab} and 987,60±77,58^{ab} for *A. conyzoides* and *V. amygdalina* respectively) with no significant difference (P>0,05) between the two plants. However there was a significant difference (P<0,05) between the weight gain of groups treated with 3 (812,80±57,92^{cd} and 884,30±122,78^{bc}for *A. conyzoides* and *V. amygdalina* respectively) and 1, 5 g/l (594,13±57,79^{e} and 719,20± 70,26^{de}for *A. conyzoides* and *V. amygdalina* respectively). All treated groups showed better FRC as compared to the infected non-treated group (P<0,05). Among the plant extract treated groups, the best FRC was recorded in chicks treated with 6 g/l and 3 g/l with no significant difference for the two plant (P>0,05). However, there was a significant difference in FRC of groups treated with 1, 5 g /l (3, 81±1,20$^{\mathbf{b}}$ and 2,90±0,57$^{\mathbf{bc}}$respectively for *A. conyzoides* and *V. amygdalina)*. The highest

FRC was observed in non-treated groups (6,47) while the least was in non-infected and non-treated groups (1,81).

3-2-2 – Haematological parameters

The haematological parameters for *E. tenella*-infected chicken treated with ethanolic extracts of *A. conyzoides and V. amygdalina* are presented in **Table 3**. It can be seen from this table that, the mean RBC, Hb and PCV after treatment with the various concentrations of ethanolic extracts was significantly (P<0,05) higher than the untreated group.

4- Discussion

This study showed that extracts from both plants significantly reduced the oocysts count of *E. tenella*-infected chickens similarly to the reference drug (Anticox) when compared to the control non-treated chickens. Nweze and Obiwulu (2009) also observed that ethanolic extracts of *A. conyzoides* reduced the faecal oocysts output of the infected birds steadily until it got to zero after 18 days of treatment. On the contrary, AL-Fifi (2007) reported that the powder of *V. amygdalina* leaves reduced the OPG to only 35%. These authors proposed that the anticoccidial effect of these plants could be attributed to its antioxidant properties and that the antioxidants constituents are flavonoids and vernosides B_1 for *A. conyzoides* and *V. amygdalina* respectively. Khaliq *et al.* (2015) observed that the use of antioxidant rich plant extracts has shown comparable results to synthethic drugs against coccidiosis. Indeed, the coccidian parasite induced host cell destruction and is associated with oxidative stress and lipid peroxidation; the antioxidants which have the ability to neutralize reactive oxygen species (ROS) are protective due to their ROS-scavenging ability (Wang *et al*. (2016). Allen and Danforth, (1998) also reported that, the use of antioxidants from natural sources in the poultry industry can help in restoring the balance of oxidants/antioxidants, leading to an improvement in birds infected with coccidiosis. These authors also showed that, plant with antioxidants properties could reduce the severity of *Eimeria* infection by ameliorating the degree of intestinal lipid peroxidation. Moreover, it can be emphasized that plant extracts inhibited development of *Eimeria* life cycle in the host cell before oocysts are released in host faeces, thus ultimately decreased *Eimeria* oocyst excretion and severity of infection (Dkhill *et al*., 2011).

We also noticed that, in infected-non treated control group, the oocyst numbers rose rapidly to attend the peak count by day 10 post-infection after which it reduced progressively throughout the duration of the study. Similarly, Mpoame Mbida *et al.* (2003) observed the same trend in a control non-treated group. This could be the result of *Eimeria* resistance leading to the natural phenomenon of self-deparasitism (Lévine (1985).

The improvement of weight gain correlated with the lower FCR observed in treated group. Similar findings have been reported by Nweze and Obiwulu (2009), AL-fifi, (2007), Adel Feizi *et al*. (2014), Nghonjuyi *et al*. (2015), Wang *et al*. (2016), Gotep *et al*. (2016). According to Ola-Fadunsin *et al*. (2013), the improvement in total body weight could be attributed to the decrease in the number of *Eimeria* oocysts in the caeca. Loddi *et al*. (2002) also suggested that the better FCR in treated groups may be due to the healthy intestinal tract of the bird and better nutrient utilization. Moreover, Gotep *et al*. (2016) opined that the improvement of growth parameters observed in extracts treated groups when compared to the infected non-treated groups is possibly due to the inhibition of inflammation in the intestinal mucosa which is suggestive of an increased nutrient absorption across the intestinal wall. However, Adel Feizi *et al*. (2014), Nghonjuyi *et al*. (2015) and Wang *et al*. (2016) suggested that the improvement of these parameters in the extracts treated groups could be due to the antimicrobial properties of the extracts.

There was a significant increase ($P<0,05$) in haematological parameters in treated groups when compared to the infected-non treated groups. This could be attributed to the daily reduction in the oocysts shed in feces as reported by Ola-Fadunsin *et al*. (2013).

Moreover, according to Gotep *et al*. (2016), the increase in RBC and haemoglobin concentration is indicative of the hematopoiesis promoting ability of the extracts, which is beneficial since the *Eimeria* parasite in the intestinal epithelium causes bloody diarrhoea and consequently anaemia. In fact, Ita *et al*. (1991) suggested that ethanolic extracts of *A. conyzoides* possessed haematopoietic potentials and could possibly remedy anaemia. Also, Osho *et al.* (2014) suggested that the higher values of haematological indices in groups treated with extract of *V. amygdalina* can be due to their anti-inflammatory potentials.

Conclusion

The findings of the present study suggests that ethanolic extracts of *A. conyzoides* and *V. amygdalina* when added in drinking water for five consecutive days could be considered as best substitute to anticoccidial drugs for the control of avian coccidiosis. However, further *in vivo* toxicity studies are recommended to investigate the potential presence of toxic effects in order to determine the minimum non-lethal doses for the treatment of coccidiosis.

5- References

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Table 1: Mean Oocyst per grams of faeces and oocysts reduction rate of broilers experimentally infested with *E. tenella* oocysts and treated with ethanolic extracts of *A. conyzoides* and *V. amygdalina*

		Mean oocysts count **(x 10^2**)					**Oocysts reduction rate**
Treatments	**Concentration (g/l)**	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	
	6	140,00±91,86^{a}	62,00±49,78^{cd}	6,80±3,96^{d}	0,40±0,55^{c}	0,00±0,00^{c}	100,00±0,00^{a}
Ageratum	3	143,80±40,47^{a}	71,00±47,86^{cd}	46,80±32,07^{bc}	3,00±2,12^{c}	1,00±1,22^{c}	99,05± 1,52^{a}
conyzoides	1, 5	145,20±31,63^{a}	135,00±34,22^{ab}	65,00±30,93^{b}	22,80±32,17^{b}	8,40±9,24^{b}	93,54±7,84^{a}
	6	141,80±9,28^{a}	41,80±31,60^{de}	9,00±5,79^{d}	0,80±1,10^{c}	0,00±0,00^{c}	100,00±0,00^{a}
Vernonia	3	147,20±64,16^{a}	93,40±50,57^{bcd}	25,00±13,86^{cd}	4,80±5,07^{c}	2,40±3,36^{c}	98,02±2,83^{a}
amygdalina	1, 5	139,75±34,39^{a}	106,75±13,89^{abc}	49,25±25,57^{bc}	8,25±3,77^{bc}	4,75±2,22^{bc}	96,12±2,58^{a}
INT	Tween 80 0, 2%	136,20±24,80^{a}	154,40±2,96^{a}	140,60±2,96^{a}	117,80±6,57^{a}	100,00±3,08^{a}	25,09±10,32^{b}
ITA	0,33	147,40±76,10^{a}	50,60±71,41^{cde}	6,80±5,89^{d}	0,20±0,45^{c}	0,00±0,00^{c}	100,00±0,00^{a}
NINT	/	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00	100,00±0,00

Values are Mean ±SEM. For the column, values carrying the same superscript letter are not significantly different at P>0, 05. INT: Infected non-treated, ITA: Infected and treated with Anticox: NINT: Non-infected-non treated.

Table 2: Mean total feed consumed, Total weight gain and feed conversion ratio of chickens treated with ethanolic extract of *A. conyzoides* and *V. amygdalina*.

		Host growth parameters		
Treatments	**Concentration (g/l)**	**TFI**	**TWG**	**FCR**
	6	2086,28±148,47	1008,90±111,90^{ab}	2,08±0,11^{c}
Ageratum conyzoides	3	1935,00±316,60	812,80±57,92^{cd}	2,39±0,54^{c}
	1, 5	2127,63±231,69	594,13±57,79^{e}	3,81±1,20^{b}
	6	2246,40±160,57	987,60±77,58^{ab}	2,28±0,20^{c}
Vernonia amygdalina	3	2000,16±439,69	884,30±122,78^{bc}	2,34±0,73^{c}
	1, 5	2061,48±308,89	719,20± 70,26^{de}	2,90±0,57^{bc}
INT	Tween 80 0, 2%	2155,38±404,15	355,38±94,65^{f}	6,47±2,76^{a}
ITA	0,33	2279,00±228,16	1018,13±90,71^{ab}	2,25±0,31^{c}
NINT	/	1983,84±357,35	1105,90±101,10^{a}	1,81±0,50^{c}

Values are Mean ±SEM. For the column, values carrying the same superscript letter are not significantly different at P>0, 05. INT: Infected non-treated, ITA: Infected and treated with Anticox, NINT: Non-infected-non treated, TFI: Total Feed Intake, TWG: Total Weight Gain, FCR: Feed Conversion Ratio.

Table 3: Effect of ethanolic extract of *A. conyzoides* and *V. amygdalina* on RBC, Hb and PCV of chickens infected with *E. tenella* oocysts

		Haematological parameters		
Treatments	**Concentration (g/l)**	**RBC**	**Hb**	**PCV**
Ageratum conyzoides	6	4,05±0,70[a]	23,60±1,74[a]	55,47±10,54[a]
	3	4,05±0,29[a]	22,13±4,84[a]	55,87±4,77[a]
	1, 5	3,72±0,21[ab]	22,13±2,05[a]	52,53±2,57[a]
Vernonia Amygdalina	6	4,36±0,29[a]	24,67±1,22[a]	61,07±7,43[a]
	3	4,65±0,79[a]	23,47±2,20[a]	57,47±4,03[a]
	1, 5	4,05±0,70[a]	22,40±1,06[a]	54,93±6,43[a]
INT	Tween 80 0, 2%	2,80±0,80[b]	11,07±2,34[b]	26,00±5,64[b]
ITA	0,33	4,55±0,58[a]	24,67±2,34[a]	63,47±7,86[a]
NINT	/	4,39±0,57[a]	21,60±0,40[a]	57,73±3,71[a]

Values are Mean ±SEM. For the column, values carrying the same superscript letter are not significantly different at P>0, 05. INT: Infected and non-treated, ITA: Infected and treated with Anticox, NINT: Non-infected-non treated, RBC: Red Blood Cell, Hb: Hemoglobin, PCV: Packed Cell Volume