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Comparative efficacy of amprolium and crude extracts of *Prosopis africana* leaf for the treatment of West African dwarf goats infected with coccidial oocysts

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Abstract

This study aimed at evaluating the comparative efficacy of amprolium and crude extracts of Prosopis africana (Iron tree) leaves in the treatment of West African dwarf goats infected with coccidial oocysts. The efficacy of amprolium, aqueous and methanolic extracts were tested using a total of 20 West African dwarf goats (WAD) of both sexes. The goats were randomly divided into five groups comprising of four goats each for amprolium, aqueous and methanolic extracts. Coccidial oocyst count reduction per gram of faeces was determined. Data collected were subjected to one-way analysis of variance (ANOVA) procedure in SPSS software version 22. The ever increasing cases of coccidiosis in goat's especially young goats have continued to be a source of worry for rural livestock farmers. This is so because they could not afford the available synthetic anticoccidial due to high cost and the chemical residue concerned in animal products and environmental pollution have awaken interest in medicinal plants as an alternative anticoccidial. The result revealed the presence of flavonoids, Saponins and tannins in all the extracts but absence of alkaloids in fresh and dried aqueous extracts except methanolic extract and also absence of glycosides in all the extracts. Maximum percent oocyst count reduction of 93.54% and 99.38% on day six and day eight post treatments (PT) were recorded in goats treated with 100mg/kg b.w. of the fresh leaves aqueous extract and amprolium respectively. It was concluded that the leaves extracts of Prosopis afracana had anticoccidial efficacy that is comparable with the amprolium.

Keywords: Goats, *Prosopis africana,* coccidial oocysts, aqueous and methanolic extracts

Efficacité comparée de l'amprolium et des extraits bruts de feuille de Prosopis africana pour le traitement des chèvres naines d'Afrique de l'Ouest infectées par des oocystes coccidiens

Résumé

Cette étude visait à évaluer l'efficacité comparative de l'amprolium et des extraits crus de feuilles de Prosopis africana (arbre de fer) dans le traitement des chèvres naines d'Afrique de l'Ouest infectées par des oocystes coccidiaux. L'efficacité des extraits d'amprolium, aqueux et méthanolique a été testée sur un total de 20 chèvres naines d'Afrique de l'Ouest (NAO) des deux sexes. Les chèvres ont été réparties au hasard en cinq groupes comprenant chacun quatre chèvres pour les extraits d'amprolium, aqueux et méthanolique. La réduction du nombre d'oocystes coccidiens par gramme de matières fécales a été déterminée. Les données recueillies ont été soumises à une procédure d'analyse de variance à un facteur (ANOVA) dans la version 22 du logiciel SPSS. En effet, ils ne pouvaient pas se permettre l'anticoccidien synthétique disponible en raison du coût élevé et les résidus chimiques concernés dans les produits animaux et la pollution de l'environnement ont suscité l'intérêt pour les plantes médicinales comme anticoccidien alternatif. Le résultat a révélé la présence de flavonoïdes, de saponines et de tanins dans tous les extraits mais l'absence d'alcaloïdes dans les extraits aqueux frais et séchés à l'exception de l'extrait méthanolique et également l'absence de

glycosides dans tous les extraits. Le pourcentage maximum de réduction du nombre d'oocystes de 93,54 % et de 99,38 % au jour six et au jour huit après les traitements (AT) a été enregistré chez les chèvres traitées avec 100 mg/kg de poids corporel de l'extrait aqueux de feuilles fraîches et de l'amprolium respectivement. Il a été conclu que les extraits de feuilles de Prosopis afracana avaient une efficacité anticoccidienne comparable à celle de l'amprolium.

Mots-clés : Chèvres, Prosopis africana, oocystes coccidiens, extraits aqueux et méthanoliques

Introduction

Coccidiosis is a universal problem in small ruminant which if uncheck can be the cause of high mortality in the young goats (Uzal *et al.,* 2016). Coccidiosis in small ruminant especially goats is a disease of high economic importance which may be associated with high economic losses, particularly in intensive system of management (Faizal and Rajapakse, 2001). Such economic losses are low growth rate, decrease in productivity, electrolyte loss due to diarrhea, mortality and the cost of treatment (Chartier and Paraud, 2012). Coccidiosis treatment in domestic animals is commonly based on the use of synthetic anticoccidial (Costa *et al*., 2006; Jabbar *et al*., 2007) substances. However, the current efficacy of these drugs has been reduced because of frequent used and the development of resistant coccidial strains (Artho *et al.,* 2007; Behnke *et al.,* 2008). Also the high cost of these drugs, the chemical residue concerned in animal products and environmental pollution have awaken interest in medicinal plants as an alternative anticoccidial (Bizimenyera *et al.,* 2006). *Prosopis africana is* a tree that is characterized by a very hard wood, small pointed leaflets, dark rouge bark, sausage shaped fruits and pale trooping foliage. Knowing by local names *Kaki-gbagi* (Eggon), *kiriya* (Hausa), *Ayan* (Yoruba), *Okpei* (Igbo). It is a medium size tree of up to 40-60 feet height and often branching very low down. The use of plant parts for treatment of internal parasites of animals has long been in existence and is widely documented in most developed countries (Sulaiman *et al.,* 2005). Those ancient indigenous practices were discovered by a series of trial and error, which then could not be substantiated by proven scientific theories (Fajimi and Taiyo, 2005). Use of plant extracts in animals has advantages that make them attractive for use in developing countries such as low cost, access to large amounts of raw materials and easy integration into traditional cultural practice. However, scientific validation of these traditional treatments is lacking (Williams *et al.,* 2014).

Materials and methods

Study area

The study was conducted at Livestock Teaching and Research Farm of the Faculty of Agriculture, Shabu-Lafia Campus, Nasarawa State University Keffi.The Study area falls within Guinea Savannah, particularly on sandy-clay soil of Nigeria. It lies between latitude 8.5583653^{0}CNorth and longitude 8.5513946^{0}C East. It has a climate typical of the tropical zone because of its location. It has a temperature of mostly between 27^{0}C (81.25^{0}f) to 31^{0}C. Broken clouds: wind 2.11 m/s, humidity 77%, cloudiness 78, pressure 1015.0 hpa in September.

Plant collection and authentication

The leaves of *prosopis africana* (Iron tree) were collected within Lafia Metropolis. The leaves were dusted and washed to remove dirts. The samples were taken to the herbarium of the Federal College of Forestry Jos, Plateau State where it was identified, authenticated and voucher number was given FHJ 304.

Processing and extraction procedure of the leaves

The processing and extraction procedure of the plant leaves were carried out at the Faculty of Agriculture Research laboratory, Nasarawa State University Keffi. Fresh leaves samples were pounded, mixed with distilled water, squeeze and sieved to obtain Fresh Leaves Aqueous Extract (FLAE). A second batch of fresh leaves was shade dried for two weeks and the dried leaves were pounded using mortar and pestle. It was then divided into two portions. The first portion was extracted with distilled water and obtained the dried leaves aqueous extract (DLAE) while the second portion was extracted using methanol. One thousand gram (1000g) of the dried leaves powder was packed into a clean plastic rubber container. 1.5 liter of methanol was added to the sample and allowed to remain for 48 hours for extraction. The extract was collected using Soxhlet extractor and concentrated by drying in the oven at 45°C to remove all the remaining solvent. The extracts were stored at 4°C and continued using it until the end of the experiments

Phytochemical screening of the leave extract

Qualitative phytochemical analyses of the fresh and dried leaves aqueous extract as well as methanolic leaves extract (MLE) were carried out according to established standard methods (Harborne, 1984). And subjected to qualitative phytochemical screening for: Saponins, Alkaloids, Tannins, Flavonoids and Glycosides.

Source of coccidial oocyst

The goats were naturally infected with the mixed species of coccidialoocyst which was tested and confirmed their presence using the Mac Master Technique.

Sources of experimental animals and their management

Apparently healthy West Africa Dwarf (WAD) Goats of both sexes weighing between 9-12kg were obtained from Faculty Research Farm and Lafia Cattle Market (Shinge) were used for the experiment. The pens were disinfected two-weeks prior the arrival of the goats. The goats were allowed to stay for two weeks for acclimatization on concrete floored in separate pens and were fed with crop residue and Water *ad-libitum.*

Experimental procedure

Experiment 1

A total of 20 West Africa Dwarf (WAD) goats of both sexes were used for the experiment. The Goats were randomly divided into five groups comprising four goats each. The goats were infected with mixed species of coccidial oocyst and all goats were monitored for clinical signs of coccidiosis before treatment commenced. Goats in Treatment 1 (T_1) were infected and left untreated (only received distilled water) to serve as negative control. Those in Treatment 2 (T_2) were infected and treated with Aprolium (Amprolium 250 WSP, Kepro® B.V., Holland) at 100mg/kg body weight to serve as positive control, Treatment 3 (T_3) goats were infected and treated with fresh leaves aqueous extract (FLAE), Treatment 4 (T_4) goats were infected and treated with dried leaves aqueous extract (DLAE) and Treatment 5 (T_5) goats were also infected and treated with methanolic leaves extract (MLE). T_3, T_4 and T_5 were each treated at a dose of 100mg/kg body weight (B.W.). The methanolic leaves extract was dissolved in about 10ml of distilled water for easy administration. Faecal oocyst count reduction (FOCR) was monitored for eight days using Mac Master Technique by counting the number of oocyst present in the feacal sample of each goat, starting from day 0 pre-treatment and at day 2, 4, 6 and 8 post-treatment (PT). All treatments lasted for five days period. Clinical signs due to side effect of the leaves extract were observed.

Data collection

Oocyst count

Fresh faecal sample of each goat was collected directly from the goat's rectum into labeled sterile universal bottles for identification were taken immediately for analyses to evaluate the presence of coccidial oocyst before and after the administration of the leaves extract, using Mac Master Techniques. The goats were weighed before and after the treatment.

Quantitative estimation of oocyst (Mc Master counting technique). The following equipment were used: Microscope, Mc Master Slide, tally counter, saturated salt solution, spatula, scale, beaker, mortar and pistle, coffee stumer, pusteur pipette and small bowls.

4g of faeces fresh were measured and crush in a mortar and turned into a beaker, 56ml of saturated salt solution was added in the beaker and pass through a coffee strainer into a small bowl, gently swirl fluid to evenly suspend the oocyst, withdraw suspension from the middle of depth of fluid with a posteur pipette and fill the two chambers of a Mc Master Slide, allow slide to stand for about 5 minutes then count all the oocyst under both ruled squares (Examine with ×10 objectives lens) and calculated using: Number of oocyst × 50 = Oocyst per gram (opg) of faeces counted.

Experimental design and statistical analysis

The design of the experiment was completely randomized design (CRD), a random sampling technique was considered for the assembling and distribution of the goats. Data collected were subjected to one-way analysis of variance (ANOVA) of the SPSS statistical package version 22. Where significance differences exist, means were separated using Duncan Multiple Range Test (DMRT) of the same package, at the confident level of 5% level of significance.

Results

Extraction and phytochemical screening

The phytochemical screening of the leaves extracts is presented in Table 1. The result revealed the presence of tannins, saponins, flavonoids in all the extracts but absence of alkaloid in fresh and dried aqueous extracts except in methanolic extract and also absence of glycosides in all the extracts.

Table 1: Phytochemical screening of iron tree (*Prosopis Africana*) leaves extracts

SAMPLE CODE	TANINS	SAPONINS	FLAVONOIDS	ALKALOIDS	GLYCOSIDES
Aq-FE	+	+	+	_	_
Aq-DE	+	+	+	_	_
MCE	+	+	+	+	_

Key: + = Present, - = Absent, Aq= Aqueous, FE = fresh extract, DE = dried extract and MCE = Methanol Crude Extract.

Effect of iron tree (Prosopis Africana) leaves extracts on oocyst count per gram of faeces (efficacy study)

The effects of *Prosopis Africana* leaves extracts on oocyst count per gram of faeces in West African Dwarf (WAD) goats infected with natural mixed species of coccidial oocyst using different processing methods is presented in Table 2. From the result obtained, different processing methods had no significant (P>0.05) effects on the oocyst counts reduction across all the treatment except at day six post treatment (PT). However, treatments 2 and 3 (positive control and FLAE) showed the best percentage oocyst reduction per gram of faeces at days eight and six PT (99.38%) and (93.54%). There were no any clinical effects/side effects that manifested on the goats after taking the extracts.

Table 2: Effects of *Prosopis africana* leaves extracts on oocyst per gram (OPG) of faeces in WAD goats infected with natural mixed species of cocci dial oocyst using different processing methods

Treatment	Pre treatment (Day 0)	PT (Day 2)	PT (Day 4)	PT (Day 6)	PT (Day 8)
Control (-ve)	1250.00±405.17 (0%)	3887.50±2383.57	1662.5±607.72	19350.00±8130.73^{b}	19700.00±11337.13
Positive (+ve) Amprolium 100mg	6062.50±5979.18 (0%)	2050.00±1528.34 (66.19%)	100.00±67.70 (98.35%)	225.00±178.54^{a} (96.29%)	37.50±23.94 (99.38%)
FALE (100mg)	12375.00±11927.59 (0%)	55537.50±54488.43	10037.50±9754.81 (18.89%)	800.00±467.71^{a} (93.54%)	1425.00±1210.80 (88.48%)
DALE (100mg)	2525.00±2208.93 (0%)	31625.00±24626.79	40412.50±32473.47	3262.50±1437.93^{a}	23812.50±18319.89
MLE (100mg)	1575.00±349.11 (0%)	8962.50±7724.35	50437.50±49191.39	650.00±348.21^{a}(58.73%)	412.50±216.39 (73.81%)
LOS	NS	NS	NS	*	NS

Key: -ve= Negative, +ve= Positive, FALE= fresh aqueous leaves extract, PT= Post treatment, DALE= Dried aqueous leaves extract, LOS= Level of significant and NS= Not significant. Values in the same column with different letters superscripts are significantly different.

Discussion

This study revealed the anticoccidial activities of the various processing method of leaves extracts of *Prosopis africana.* The result showed that there was significant ($P \leq 0.05$) effect of *prosopis africana* leaves on the oocyst count as demonstrated in the treated group that received dose of 100mg/kg body weight (B.W.) was comparable with Amprolium at the dose of 100mg/kg B.W. which was similarly reported by Constable *et al. (*2012). The reduction in oocyst count per gram of faeces observed in treated groups could be attributed to the present of some phytoconstituents in the leaves extracts against the mixed species of parasites (Jackson *et al.,* 2015). Saponins was reported to restrain parasites membrane and cholesterol level, thereby altering the integrity of the parasite membrane which results to loss of homeostasis and eventual death of the organism (Wang *et al.,* 2010). The leaves of *Prosopis glandulosa* have been reported to have efficacy against parasites (Rahman *et al.,* 2011). The observed increase in the oocyst load in some days contrary to the expected decrease after treatment could be attributed to the mode of action of the various phytoconstituents present in the leaves extracts. It might also be due to the resistance of some species of the Coccidial oocyst to the phytochemical which allow them to multiply and thereby causing an increase in the oocyst load.

Conclusion

This study showed that the leaves extracts of *prosopis afracana* had ananticoccidial efficacy that is comparable with the synthetic/conventional drug (Amprolium), and could be used by the farmers as alternative anticoccidial drug to reduce cost of conventional drug and the effects of chemicals residues in animal products, such as meat and milk. Since the plants can be found almost everywhere in our locality and easy to process, and the result also revealed the safety of the extracts to be used without causing any side effects to the goats. Procedure for the processing should be strictly adhered to obtain quality and accurate result at the course of treatment.

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