



Effect of *Euclea divinorum* Aqueous Leaf Extract in the Treatment of Coccidiosis in Broilers at the Poultry Unit, Federal College of Forestry Jos, Plateau State, Nigeria

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Abstract

The study was carried out to analyze the effect of *Euclea divinorum* Aqueous Leaf Extract in the Treatment of Coccidiosis in Broilers at the Poultry Unit, Federal College of Forestry Jos, Plateau State, Nigeria. Sixty (60) birds were experimentally infected with (5%, 10% 20% and 0%) solution of *Euclea divinorum*. The birds were divided into four groups T_1 , T_2 , T_3 , and T_4 , with three (3) replicates each. Duncan count was carried out at the end of the experiment for scoring of hemorrhage and data analysis using ANOVA. The phytochemical analysis of *Euclea divinorum* indicated the presence of Alkaloids, Flavonoids, Saponins, Tannins, Phenol, Resin, Glycosides and Steriodal ring; which have been reported to have a retrogressive effects on micro-organisms. The chemical constituents of the plant extract indicated positive signs; however, Resin had a negative sign. The ANOVA result revealed significant differences among the different levels of inclusion; there is a significant difference at 5% ($p > 0.05$) level of probability in the birds treated with different levels of concentration of *Euclea divinorum* aqueous leaf extract. Treatments (T_1), (T_2), (T_3) and (T_4) reported mortality rates of 13.3% (2), 6.7% (1), 66.7% (10) and 80% (12), respectively, with gross mortality of twenty-five (25) birds. From the study, it is suggested that *Euclea divinorum* has great potentials in being used to control coccidiosis in broilers. Cultivation of *Euclea divinorum*; more support for research on using plant extracts for poultry disease control, subsidized vaccines and medical supplies, improved extension services for poultry farmers are strongly recommended.

Keywords: Broilers; Coccidiosis; *Euclea divinorum*; Leaf Extract; Treatment

Introduction

Poultry production is one essential part of agriculture that serves as a rich source of animal protein which impacts cannot be over-emphasized in human nutrition. The main source of animal protein is the livestock industry in Nigeria. Over the years, the contributions of livestock subsector to gross domestic product (GDP) have decreased from 5.61% in 1960 to about 2.64% in 2010 [1]. Livestock production constitutes an important component of the agriculture economy in developing countries and it is an instrument of socio-economic change, improving income and quality of rural life in Nigeria [2]. Poultry disease remains one of the major

threats to boosting poultry production in Nigeria. Parasitic diseases are of particular importance because of their high incidence in poultry occasioned by the tropical environmental conditions under which the farmer operates [4]. Coccidiosis is one of the important diseases of poultry worldwide [3] and is cause by apicomplexan parasites of genus *Eimeria* of which there are seven species affecting chicken. The disease is characterized by marked morbidity, mortalities and reduction in productivity and feed conversion efficiency of affected chicken [5]. It is a significant problem to the poultry industry in Nigeria and throughout the world and it is responsible for 6-10% of broiler mortalities [6]. The plant *Euclea divinorum*

(Magic Guarri or Diamond-leaves) is of the family *Hiern Ebenacea*. *Euclea* is a tropical small tree or shrub growing up to 6-18 meters tall found in eastern and southern Africa and now commonly in Nigeria. It often branches from the base. The bark is grey and smooth but becomes cracked as the tree gets old. Occasionally the plant is used as ornamental, shade tree and medicinal purposes [6]. The fruit is taken as a mild laxative but can have a strong purgative action. In traditional medicine, the root extracts and dried powdered root are used for the treatment of gastro-intestinal disturbance, cancer, miscarriage and jaundice. The leaves are used to treat diarrhoea [7]. Broilers tend to suffer from nutritional deficiency and other diseases, which lower their performance and productivity and also due to high prices of drugs and its toxicity; there is a need to identify alternative means of managing these diseases notably the use of plant-like *Euclea divinorum* which has a medical value that may help in the treatment of coccidiosis in a poultry [8]. Coccidiosis causes huge economic losses in poultry and this includes the cost of treatment of birds; reduced productivity and losses due to mortality in birds [9]. The intensive use of anti-coccidian drugs has led to the development of resistance and such products have the disadvantage of producing adverse effects [10]. Coccidiosis remains of the most common disease of the poultry industry all over the world [11]. Particularly it constitutes an important wet season hazard to poultry farmers in Nigeria [12]. Coccidiosis caused by *Eimeria* species results in huge economic losses due to bird mortality [13]. Since the introduction of various anti-coccidiosis and vaccines, however, medication with these drugs have been fairly effective in preventing a various outbreak of disease, the reason due to the emergency of resistant strains of coccidian [7]. Besides the scarcity of livestock drugs, access to anti-coccidiosis drugs is difficult, especially to rural poultry farmers [11]. This, therefore, necessitated the need to look into other alternative ways of controlling this disease, notably the use of herbs or plant extracts such as *Euclea divinorum*, which has been acclaimed both scientifically [8] and traditionally to have curative abilities for several diseases of both man and animal. The use of medical plants, either alone or in the group (combination) as possible therapeutic measures has become a subject of active scientific investigation [14]. Medical plant products (such as *Euclea divinorum*) are in high demand for their medicinal value. The main objective of this study was to determine the effect of *Euclea divinorum* leave extract on birds experimentally infected with coccidiosis, while the specific objectives were to;

- Identify the phytochemical properties of *Euclea divinorum*;
- Determine the effect of treatment of birds experimentally infected with *Eimeria tenella* using extracts of *Euclea divinorum*; and
- Estimate the mortality of birds at different concentration levels of *Euclea divinorum*.

Research hypothesis

H₀: There is no significant difference in the treatment and mortality of broilers infected with *Eimeria tenella* (coccidiosis) using extracts of *Euclea divinorum* (diamond leaves).

Methodology

Study area

The study area is in Federal College of Forestry Jos, Plateau State, Nigeria. The school is situated in Jos-North Local Government Area (LGA); located along Bauchi road opposite Bauchi Park, with coordinates between latitude 8° 30 and 10° 30N and longitude 8° 20 and 9° 30E and a land area of about 9,400 km². It has an average elevation of about 1,250 meters above sea level and stands at the height of about 600 meters above the surrounding plains [1]. Plateau state is characterized by a near temperature climate on the Jos Plateau and a hot and humid climate on its lower parts. Generally, weather conditions are warmer during the rainy season (April-October) and much colder during the harmattan period (December-February). The main annual temperature in the state ranges between 20A°C and 25A°C, while the main annual rainfall figures range from 131.75mm in the southern part to 146mm on the Jos plateau. Plateau state lies within the northern guinea savannah zone, with vegetation consisting mainly of short trees and grasses [1]. Near some villages is thick hedges of cacti, which have been planted around the household farms or compound land. Fringing woodland or gallery forests can be found around some river valleys.

Materials

Plant materials

The green leaves of *Euclea divinorum* were collected at the Federal College of forestry, Jos. The leaves were air-dried at room temperature, grinded into powder and stored in a cool dry place.

Preparation of aqueous extract of *Euclea divinorum*

Three (3) separate concentrations of the aqueous leaf extract of *Euclea divinorum* was prepared by dissolving 5g, 10g, and 20g of the powder dry leaves in 100 ml of distilled water in the conical flask and left in water for 24 hours, after which the solution were filtered using a filter paper and the filtrate was stored in a refrigerator at 4°C until use.

Experimental organisms

The experimental organism (*Eimeria tenella*) was sourced from the parasitology division on the national veterinary research institute Vom, Plateau state.

Experimental animal

Sixty broilers were purchased from Datt farm Jos, Plateau state and brood for three weeks within which routine management practice was observed. At three weeks, the broilers were divided into four (4) groups (T₁, T₂, T₃, and T₄) with three (3) replicates in each group.

Experimental infection with *Eimeria tenella* and treatment with the plant extract

Experimental Infection (Challenge) of the bird with *Eimeria tenella* was carried out at four weeks for two consecutive days; observation of clinical signs and treatment with the plant extract then commenced. To group T₁, T₂, and T₃; 1%, 5%, and 10% of the aqueous leaf extract of *Euclea divinorum* for five days, was administered respectively. To group T₄, no treatment was administered (control).

Performance parameters

The birds were observed daily for clinical signs. Morbidity and mortality were recorded daily in each sub-group. Necropsy was carried out on the birds that died on the experiment. Seven days after the challenge, the evaluation of the caecal lesion was carried out in eight chicks of each group. A lesion score was assigned ranging from 0-4: where 1 corresponds to the normal status with no gross lesion; 2 to small scatter petechiae; 3 to numerous petechiae; 4 to extensive hemorrhage that gives a dark colour to the caecal intestine. Bird mortality was given a score of 0.

Phytochemical analysis

The phytochemical analysis was carried out using standard methods in order to establish the presence and effects of the chemi-

cal substances present in the leaf extracts based on the methods of Trease and Evans (2000).

Test for alkaloid

0.5g of the plant extract, 5ml of 1% aqueous Baran Chloride (BCL) was added and stirred; the mixture was filtered and 1ml of the filtrate was treated with few drop of Drangondoffs reagent, Wagners reagent and Mayer reagent respectively. The formation of precipitates indicated the presence of Alkaloids.

Test for flavonoid

0.5g the extract was dissolved in 2ml dilute NaOH solution. A few drops of concentrate H₂SO₄ was then added to the solution to become colorless, which indicates the presence of flavonoids.

Test for saponin

0.5g plant extract was introduced into a test tube, few milliliters of distilled water was added then covered and thoroughly shaken for 1 minute; protein was observed which persisted on warming and indicative of the presence of saponin.

Test for tannin

0.5g of the plant extract was introduced to 10 mg of distilling water; stirred and filtered. Few milliliters of 5% ferric chloride solution was added to the filtrate; the presence of deep green coloration shows the presence of tannins.

Test for phenol

The aqueous extract of the plant was boiled with 1% aqueous Hydro chloride (HCL) with a very mild evidence for phenols.

Test for resins

0.5mg of the plant extract was introduced to 10ml of boiling ethanol; stirred and filtered with what-man's No.1 filter paper, the filtrate was diluted with 4ml of 1% aqueous HCL. Lack of resinous precipitates indicates the absence of resins.

Test for glycoside

0.5g of the extract was stirred with 10ml of boiling distilled water and filtered; 2ml of the filtrate was hydrolyzed with a few drops of concentrated HCL solution and 2 ml of benedict qualitative reagent; added and boiled together. A reddish-brown precipitate indicated the presence of Glycosides.

Test for steroidal ring

About 100 mg of the extract was dissolved in 2ml of chloroform; sulphuric acid was carefully added to form a lower layer. A reddish-brown precipitate was indicative of the presence of a steroidal ring.

Statistical analysis

Data were subjected to analysis of variance (ANOVA). The significant treatment effect was discussed as probability means, separated using Duncan’s new multiple range test with significance at a probability of $p < 0.05$.

Results and Discussion

Phytochemical screening

Phytochemicals Tested	Observation
Alkaloids	+++
Flavonoids	+
Saponins	+++
Tannins	++
Phenol	+
Resin	-
Glycoside	+++
Steriodal ring	++

Table 1: Photochemistry of *Euclea divinorum* Leaves.

Key: - Negative; + Mildly Present; ++ Moderately Present; +++ Highly Present.

The dried leaves extract of *Euclea divinorum* was subjected to a standard phytochemical screening for various constituents according to the method of Trease and Evans [15]. The result from the phytochemicals screening carried out (Table 1) showed that the seven (7) chemical constituents of the plant were observed to be positive. Also, there was a level of interaction among the chemical constituents; with significant treatment effects. Alkaloid was present as indicated by the formation of the precipitate. Alkaloids represent the active principle of vegetable drugs. They are alkaline in reaction and richly combine with acid farming salts soluble in water; excess alkaloids may become antagonistic [16]. Alkaloid produces analgesic, anti-inflammatory and androgenic effects which help to develop resistance against disease and endurance against

stress [17]. Test for flavonoid revealed its mild presence in the leaf extract, with significant treatment effects; the solution was not colorless as put forward by Trease and Evans [15]. Test for saponin revealed that frothing persisted and was taken as preliminary evidence for the presence of saponin [18]. Hemolysis of red blood cells around the disc after 6 hours was taken as further evidence of the presence of saponin [15]. The presence of saponin in plants has expectorant effects, which are very useful in the management of inflammation of the upper respiratory tract in addition to its cardio-tonic properties, as reported by Trease and Evans [15]. Saponins have the property of causing hemolysis of cells even at low dilution and tend to be deposited on the surface of cells with which they come in contact with and are not observed by the normal epithelium of the alimentary canal [16]. Test for Tannin revealed a deep green coloration which indicates the presence of Tannin. The faint bluish coloration after treating the filtrate with iodine solution confirmed the presence of Tannin [15]. Tannins are used in septic preparations, which produce contractions of blood vessels, stopping bleeding and have the quality of retaining hemorrhages when applied to the bleeding part [19]. Test for Phenol also revealed its mild presence in the leaf extract; with significant treatment effects. Test for glycosides showed a reddish-brown precipitate which indicates the presence of glycosides [15]. Glycosides have a tendency to block the conduction of the electrical impulse that causes contraction as it passes from the arteries to the ventricles of the heart. Cardiac glycoside also have a tendency to produce an abnormal cardiac rhythm by causing electrical impulses to be generated at points in the heart other than the normal face maker region, the cells that rhythmically maintain heartbeat. Also, test for Steroidal Ring revealed a reddish-brown precipitate; indicative of the presence of a steroidal ring in the leaf extract, which has anti-tumor effects [20].

Effect of *Euclea Divinorum* leaf extract in the treatment of broilers infected with *Eimeria tenella*

Table 2 revealed that $F_{cal} 0.55 > F_{tab} 4.07$; hence, the null hypothesis is rejected. The ANOVA result thus indicates significant difference in effects between the different levels of inclusion of aqueous leave extract of *Euclea divinorum* in the treatment of *Eimeria tenella* in broilers.

Source of variance	Sum of square	Degree of freedom	Mean square SS/df	MST MSE	Critical value 5% Tab	1%
Total	152.25	11	13.84			
Treatment	26.25	3	8.75	0.55	4.07	7.59
Error	126	8	15.25			

Table 2: Degree of Effects of *Euclea divinorum* on Broilers Experimentally infected with *Eimeria tenella*.

Source: Field Survey, 2020.

Mortality of birds at different concentration levels of *Euclea divinorum*

Treatment	Flock size	Mortality	Percentage (%)
T ₁ (5%)	15	2	13.3
T ₂ (10%)	15	1	6.7
T ₃ (20%)	15	10	66.7
T ₄ (Control) (0%)	15	12	80

Table 3: Distribution Based on the Mortality of Birds at Different Concentration Levels of *Euclea divinorum*.

Source: Field survey, 2020.

Table 3 revealed the different responses of birds infected with *Eimeria tenella* with regards to mortality rate percentage. Treatment four (T₄) has the highest mortality with 80%; treatment two (T₂) reported mortality of 66.7%, while treatments (T₁) and (T₂) reported mortalities of 13.3% and 6.7%, respectively. The implication of these results is that birds treated with 10% concentration level of *Euclea divinorum* aqueous leaf extract responded more positively to the treatment compared to other levels of concentration, hence sustained application can serve as a mitigation measure for disease outbreak and *Eimeria tenella* (coccidiosis) particularly. Therefore, *Euclea divinorum* can be used in the treatment of coccidiosis in broilers, as indicated by the results. The result indicates that the effect of the plant extract is not concentration-dependent, hence moderate application at 10% inclusion level of the aqueous extracts of *Euclea divinorum* was ideal and had significant effects in the treatment of *Eimeria tenella* (coccidiosis) in broilers as indicated by the relatively low mortality rate in T₂. It is also assumed that at a high dose of (20%), the aqueous plant extracts was toxic to the birds; indicative by the high mortality rate in T₃. Thus, there are significant differences between the different inclusion levels of the aqueous extracts of *Euclea divinorum* in the treatment of *Eimeria*

tenella (coccidiosis) in broilers; indicative by the mortality rates in the different treatments.

Conclusion and Recommendations

The study analyzed the effect of *Euclea Divinorum* Aqueous Leaf Extract in the Treatment of Coccidiosis in Broilers at the Poultry Unit, Federal College of Forestry Jos, Plateau State, Nigeria. A total of sixty (60) birds were experimentally infected with *Eimeria tenella* and then treated with aqueous leaf extract of *Euclea divinorum* at different levels of concentration. The birds were divided into four treatments T₁, T₂, T₃, and T₄ having three replicates each. The result for the phytochemical analysis indicated the presence of Alkaloids, flavonoids, saponins, Tannins, Phenol, Resin, Glycosides and Steroidal ring. The ANOVA revealed significant differences among the different levels of inclusion of the Aqueous Leaf Extract. Treatments T₂ (6.7%) reported the lowest mortality rate, while T₄ (80%) reported the highest mortality rate. The result indicates that the effect of the plant extract is not concentration-dependent; indicative by the mortality rates in the different treatments. From the result obtained, the following recommendations are made:

- Cultivation of *Euclea divinorum* be encouraged and enhanced;
- *Euclea divinorum* should be looked upon and utilized in the treating of poultry disease;
- More research work should be carried out on using different part of plant and at different concentration;
- Provision of effective vaccines and drugs should be made available for the poultry farmers at a low and more affordable cost;
- More extension services in relation to disease control should be provided for poultry farmers in the study area; and
- Research should be carried out to compare the degree of efficacy of conventional anti coccidian drugs and *Euclea divinorum*.

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