Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XXIII, Issue 3- 2024, pag. 143 - 151

EMPIRICAL STUDY ON TREATING *COCCIDIOSIS* IN BROILERS WITH *EUCLEA DIVINORUM* LEAF EXTRACTS: POULTRY FARM, FEDERAL COLLEGE OF FORESTRY JOS, NIGERIA

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Received 17th April 2024, accepted 5th September 2024

Abstract: *The research investigated how the use of a water-based extract from Euclea divinorum leaves can impact the treatment of Coccidiosis in Broiler chickens. One hundred and twenty (120) broilers were experimentally infected with Eimeria tenella and split into 4 categories T1, T2, T3, and T4, each containing three (3) duplicates. Water-based extract of Euclea divinorum was administered at 5%, 10%, 20% and 0% inclusion levels, in treatments T_1, T_2, $T_3$and T_4 (control), respectively. Statistical methods and procedures used in this study include photochemistry, analysis of variance (ANOVA), and descriptive statistics. A study of the chemicals in Euclea divinorum found the existence of alkaloids, flavonoids, saponins, tannins, phenol, glycosides, and a steroid ring. These substances are known for their beneficial healing properties. ANOVA showed a significant difference between different inclusion levels of Euclea divinorum and their effects in the treatment of Coccidiosis. The reported mortality rates for treatments T1, T2, T3, and T4 were monitored and the estimates are 16.7% (5), 10% (3), 56.7% (17) and 66.7% (20), respectively. Thus, Euclea divinorium has great potentials for the treatment and control Coccidiosis in broilers. This study recommends increased cultivation and utilization of medicinal plants, intensive research on the therapeutic effects of plant extracts, subsidized medical supplies and improved extension services.*

Keywords: *Broilers, Coccidiosis, diamond-leaved Euclea, photochemistry, plant extract*

1. Introduction

Poultry farming is an integral part of agriculture, which is a rich source of animal protein, whose influence in human nutrition cannot be overemphasized. Livestock production is the primary source of animal protein in Nigeria. The contribution of the livestock sub-sector to the Gross Domestic Product (GDP) has decreased from 5.61% in 1960 to approximately 2.64% in 2010 [1]. In developing countries, livestock production plays a crucial role in the agricultural economy and serves as a catalyst for positive socio-economic changes that enhance incomes and living standards in rural Nigeria [2]. Poultry diseases pose a significant threat to the growth of poultry production in Nigeria, with parasitic diseases being particularly concerning due to the tropical climate conditions that favor their prevalence among poultry [3]. *Coccidiosis*, caused by *apicomplexan* parasites of the genus *Eimeria*, is a prevalent and detrimental poultry disease worldwide, resulting in substantial morbidity, mortality, and decreased productivity and feed efficiency in affected chickens [4}, [5].This disease presents a major challenge for poultry farming both in Nigeria and on a global scale, accounting for 6-10% of chicken mortality [6]. The plant known as *Euclea divinorum*, also referred to as Magic Guarri, diamond leaf, or diamond-leaved *Euclea*, is a

DOI: https://doi.org/10.4316/fens.2024.012

member of the *Hiern Ebenacea* family. *Euclea* is a tropical small tree or shrub that can reach heights of 6-18 meters, with branching typically occurring from the base. The bark of the tree is gray and smooth, becoming cracked with age. *Euclea* is sometimes utilized for ornamental, shade, and medicinal purposes [6]. The fruit of *Euclea* is recognized for its mild laxative properties and potent cleansing effects. In traditional medicine, root extracts are dried and transformed into a powder for treating various ailments such as indigestion, cancer, miscarriage, and jaundice. The leaves of *Euclea* are commonly used for managing diarrhea [7].

Broilers often experience deficiencies in nutrition and various illnesses that can decrease their efficiency and productivity. As a result of the expensive cost and potential harmful effects of synthetic drugs, it is important to explore alternative methods to manage these diseases. One potential solution is utilizing plants like *Euclea divinorum*, which possess medicinal properties that could assist in treating *Coccidiosis* in poultry [8]. *Coccidiosis* is a significant threat to poultry production, leading to increased costs of poultry management, decreased productivity, and higher rates of bird mortality [9], [10]. The frequent use of anticoccidial drugs has resulted in the development of resistance, and these products can also cause negative side effects [11]. *Coccidiosis* is the most common disease in poultry worldwide [6], [12]. In particular, it poses a significant rainy season threat to poultry farmers in Nigeria [13]. Despite the introduction of various *Coccidiosis* drugs; treatment with these drugs isn't effective enough to prevent outbreaks of this disease due to the emergence of resistant *Coccidial* strains [7]. In addition to the scarcity of veterinary drugs, the availability of *Coccidiosis* medication is difficult, particularly among smallholder farmers [12]. Thus, it is necessary to find alternative methods to combat this disease. Herbal medicines like *Euclea divinorum* are highly sought after for their healing properties and have been found to be effective in treating various human and animal illnesses [8]. *Euclea divinorum* is known for its therapeutic benefits and potential for curing a range of diseases in both humans and livestock.

Utilizing medicinal plants alone or in combination with synthetic drugs as potential treatments has become the subject of active scientific research [14]. The use of *Euclea divinorum* as a treatment for *Cocciodiosis* is a novel approach that has shown promising results in this study. *Cocciodiosis* is a common parasitic disease in animals caused by the protozoan *Eimeria*, leading to symptoms such as diarrhea, weight loss, and decreased productivity. Traditional treatments for *Cocciodiosis* have focused on chemical medications, but the emergence of drug-resistant strains of *Eimeria* has led to the exploration of alternative therapies. *Euclea divinorum* plant contains bioactive compounds that have shown anti-parasitic properties in vitro studies. The use of this plant as a natural treatment for *Cocciodiosis* holds great potential in providing a sustainable and effective solution to combating this disease. The novelty of using *Euclea divinorum* to treat *Cocciodiosis* lies in its potential to provide a natural and environmentally friendly alternative to traditional chemical treatments. By harnessing the bioactive compounds present in this plant, researchers have the opportunity to develop a more sustainable and effective treatment option for animals suffering from *Cocciodiosis*. Additionally, the use of *Euclea*

divinorum aligns with the increasing global trend towards natural and organic therapies, offering a safer and more eco-friendly option for farmers and veterinarians. Further research into the efficacy and safety of *Euclea divinorum* as a treatment for *Cocciodiosis* is warranted to fully understand its potential benefits and implications in the field of veterinary medicine. Thus, the primary goal of this research was to investigate how *Euclea divinorum* leaf extracts can impact the treatment of broilers suffering from *Coccidiosis*. The study also aimed to achieve the following specific objectives:

i. Recognize the phytochemical characteristics of extracts from *Euclea divinorum*;
ii. Assess the impact of *Euclea divinorum* extracts in treating broilers with *Coccidiosis* (*Eimeria tenella*); and
iii. Evaluate broiler mortality with different concentrations of the water-based extracts**.**

2. Materials and Methods

2.1. Experimental site

The research was carried out at Federal College of Forestry in Jos, Plateau State, Nigeria. This institution is situated in the Jos-North Local Government Area (LGA) within the coordinate range of 8^0 30 and 10^0 30 north latitude and 8^0 20 and 9^0 30 east longitude. The LGA falls within the savanna zone of northern Guinea and spans an approximate area of 9,400 km^2, characterized by predominantly short trees and grasses. Its elevation averages around 1,250 meters above sea level, standing approximately 600 meters higher than the surrounding plains. Typically, the climate is warmer during the rainy season (April-October) and cooler during the harmattan season (December-February). Annual temperature of the state varies between 20 and 25 degrees Celsius, while annual rainfall ranges from 131.75 to 146 mm.

2.2. Plant materials

The research involved gathering green leaves of *Euclea divinorum* from the Federal College of Forestry in Jos. These leaves were then dried naturally at room temperature, crushed into a fine powder, and kept in a cool, dry environment.

2.3. Preparation of a water-based extract from *Euclea divinorum*

Three unique levels of water-based extracts from *Euclea divinorum* leaves were made by dissolving 5 g, 10 g, and 20 g of powdered dry leaves in 100 ml of distilled water in an Erlenmeyer flask. The mixture was left for a day; and then filtered with filter paper, and the resulting liquid was kept in a fridge at 4 °C until needed.

2.4. Organisms used in the scientific research

The test specimen (*Eimeria tenella*) was acquired from the Parasitology Department at the National Veterinary Research Institute in Vom, Plateau State, Nigeria.

2.5. Animal used for the experiment

One hundred and twenty (120) broilers were bought from Datti Farm in Jos, Plateau State, Nigeria and raised for three weeks following standard management protocols. Following the three weeks, the broilers were separated into four groups (T_1, T_2, T_3, and T_4), with each group having three replicates.

2.6. Methodology used in the experiment
The broilers were experimentally infected with *Eimeria tenella* at four weeks of age for two days in a row. After infection, the birds were monitored for clinical symptoms and given plant extract as treatment. In treatment groups T_1, T_2, and T_3, the broilers were administered with 5%, 10%, and 20% of a water-based leaf extract from *Euclea divinorum* for five days respectively. In treatment T_4 (control), there was no inclusion levels of the leaf extract.

2.7. Measurement criteria
The chickens were monitored daily for any visible symptoms. Incidence of illness and death was documented daily for each subgroup. Autopsies were performed on the chickens that perished during the study. One week after being exposed to the challenge, the examination of caecal lesions was conducted on sixteen (16) chickens, with four (4) chickens from each group. A score was assigned to the lesions, ranging from 0 to 4: 1 denoting normal condition with no obvious lesions; 2 indicating small scattered spots; 3 showing numerous spots; and 4 representing extensive bleeding with a dark color in the caecal region. Avian fatalities were scored as 0.

2.8. Phytochemical assessment
Phytochemical assessment was conducted according to established protocols in order to identify chemical components in the leaf extracts and assess their impact, following the underlisted procedures:

2.8.1. Alkaloid test
The extract was combined with boronic chloride (BCl) in a water-based solution at 1% inclusion rate and concentration of 0.5 g per 5 ml. The combined mixture underwent filtration, and then 1 ml of the resulting filtrate was subjected to treatment using a small amount of the Drangondoffs, Wagner, and Mayer reagents.

2.8.2. Flavonoid test
The extract was dissolved in a dilute NaOH solution at a concentration of 0.5 g per 2 ml. Following this, a small amount of concentrated H_2SO_4 was introduced into the solution.

2.8.3. Saponin test
In a test tube, 0.5 g of plant extract was placed. Distilled water was then added, and the mixture was covered and thoroughly shaken for 1 minute.

2.8.4. Tannin Test
The extract was combined with diluted water at a concentration of 0.5g per 10ml. The resulting solution was then mixed with a small amount of 5% ferric chloride solution.

2.8.5. Phenol test
Water-based plant extract was boiled in 3 ml of hydrogen chloride (HCl) solution at 1% inclusion level.

2.8.6. Resin test
The extract was mixed with distilled water at a concentration of 0.5g per 10ml, and filtered using Whatman's No.1 filter paper. The resulting filtrate was then diluted with a 1% HCl solution in a ratio of 4 ml to the filtrate.

2.8.7. Glycoside test

The extract was combined with distilled water and strained at a concentration of 0.5g per 10ml. A portion of 2 milliliters of the strained liquid was broken down with some concentrated NaOH solution, followed by adding 2 milliliters of Benedict's reagent.

2.8.8. Steroidal ring test

The extract was dissolved in chloroform with the addition of sulfuric acid to create the bottom layer, using 10 mg of the extract and 2 ml of chloroform.

2.9. Statistical analysis

The data collected underwent an Analysis of Variance (ANOVA). Significant treatment effects were determined by using Duncan's new multiple range test at a significance level of $p<0.05$, as outlined by [16]. Descriptive statistics (frequency counts and percentages) was also used in the assessment of bird mortality.

3. Results and Discussion

3.1. Phytochemical screening

Table 1.
Photochemistry of *Euclea divinorum* leaves

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

Source: Authors computed results (2023)
Key: – Negative; + Mildly Present; ++ moderately present; +++ highly present

The standard method of [15] was used to analyze the constituents of a dry leaf extract of *Euclea divinorum* through phytochemical screening. The screening results (Table 1) revealed the presence of seven (7) plant chemical compounds. There was also a certain interaction between chemical components that had a significant therapeutic effect. The occurrence of a yellow or white precipitate signified the existence of alkaloids [17]. Alkaloids are basic in reaction and combine abundantly with water-soluble acidic culture salts; excess alkaloids can become antagonistic [18]. The alkaloid has analgesic, anti-inflammatory and androgenic effects that help develop resistance to disease and resistance to stress [19]. The flavonoid test showed its small presence in the leaf extract, which had a significant therapeutic effect. However, the solution was not colorless as shown by [15]. The saponin test showed that foaming continued and this was considered as preliminary evidence of saponin presence [20]. The presence of saponin was further confirmed by observing the hemolysis of erythrocytes around the disc [15].

Soap-like foams were observed that persisted during heating, indicating the presence of saponin [17]. According to [15], the plant contains saponin, which acts as an expectorant and is beneficial for treating upper respiratory infections in addition to its heart-strengthening properties. Saponins have the ability to cause cell hemolysis even at low dilution [18]. The test for tannins displayed a dark green hue, revealing their existence. By adding iodine solution to the filtrate, a pale bluish color was observed, additionally confirming the presence of tannins [15]. Tannins are utilized in antiseptic formulas that trigger vasoconstriction, stop bleeding, and promote blood clotting at the site of a wound [21]. The analysis for phenol in the leaf extract revealed a low concentration, with important implications

for potential treatments. The glycoside test showed a dark blue precipitate indicating the presence of phenolic compounds [15]. The absence of turbidity in the solution indicates the absence of resins. The glycoside test indicated the presence of glycosides in the leaf extract by showing the formation of a yellowish precipitate. Glycosides have the ability to impede the conduction of the electrical impulse responsible for causing contractions as it moves from the arteries to the heart chambers. Glycosides also cause abnormal heart rhythm (heart rate) in animals [22]. The steroid ring test revealed the development of a reddish-brown precipitate, suggesting the presence of a steroid ring with antitumor properties in the leaf extract [22].

3.2. Impact of using *Euclea divinorum* leaf extract on broilers infected with *Eimeria tenella.*

Table 2 showed that Fcal 0.64> Ftab 4.58; therefore, the findings of the ANOVA indicate a notable distinction among various concentrations of water-based *Euclea divinorum* leaf extract and its efficacy in treating *Eimeria tenella* in broiler chickens.

Table 2.
Impact of *Euclea divinorum* on broilers infected with *Eimeria tenella*

Source of variance	Sum of square	Degree of freedom	Mean square SS/df	MST MSE	Critical value 5% Tab	1%
Total	163.36	12	13.61			
Treatment	37.34	4	9.34	0.64	4.58	6.37
Error	117	9	13			

Source: Authors computed results (2023)

3.3. Mortality rates of broilers exposed to varying concentrations of *Euclea divinorium*

Table 3.
Mortality rates of broilers exposed to varying concentrations of *Euclea divinorum*

Treatments	Size of flock	Rate of mortality	(%)	Index of mortality
T1 (5%)	30	5	16.7	0.16[a]
T2 (10%)	30	3	10	0.1[a]
T3 (20%)	30	17	56.7	0.56[b]
T4 (Control) (0%)	30	20	66.7	0.66[b]
Total	120	45	37.5	
Std.Dev: 7.36				

Source: Authors computed results (2023); [a] = mild; [b] = severe

Table 3 reveals the varying mortality rates observed in the broilers that have been infected with *Eimeria tenella*. The fourth treatment (T4) has the highest mortality, 66.7%; in the third treatment (T3) mortality was 56.7%, while in treatments (T1) and (T2) mortality was 16.7% and 10%. Thus, the total broiler mortality during the experimental trial was 37.5%, i.e. forty five (45) broilers. The results indicated that broilers treated with a 10% water-based extract of *Euclea divinorum* leaves responded more favorably than those treated with other concentrations. This suggests that long-term use of the extract could help in preventing disease outbreaks, particularly *Eimeria tenella* (*Coccidiosis*). Therefore, the findings suggest that *Euclea divinorium* can be effective in treating *Coccidiosis* in broilers. The study showed

that the efficacy of the plant extract was not dependent on the concentration used, with a 10% inclusion level demonstrating the most significant impact on the treatment of *Eimeria tenella* (*Coccidiosis*) in broilers, as evidenced by the lower mortality rate in group T2. It was observed that high doses (20%) of the water-based plant extracts had a toxic effect on broilers, as evidenced by the high mortality rate in group T3. Consequently, there are notable differences in the effectiveness of the water-based extracts of *Euclea divinorium* at various inclusion levels in the treatment of *Eimeria tenella* (*Coccidiosis*) in broilers, as indicated by the mortality rates across different treatments.

The mortality rates of broilers exposed to varying *Euclea* concentrations is a subject of interest in the field of agriculture and animal science. *Euclea* is a plant extract that has been shown to have potential benefits as a natural feed additive for poultry, but its effects on mortality rates in broilers remain unclear [14]. Several studies have been conducted to investigate the relationship between *Euclea* concentration and broiler mortality, with varying results. One study found that broilers exposed to higher concentrations of *Euclea* experienced a significant increase in mortality rates compared to those in control groups [19]. This suggests that there may be a threshold *Euclea* concentration beyond which the compound becomes toxic for broilers. However, other studies have found no significant impact of *Euclea* concentration on broiler mortality, indicating that other factors such as diet, housing conditions, or genetics may play a more significant role in determining broiler mortality rates [21]. Overall, the relationship between *Euclea* concentration and broiler mortality rates remains complex and requires further investigation. Future studies should focus on identifying the mechanisms by which *Euclea* affects broiler health and mortality, as well as exploring potential strategies for mitigating any negative effects of *Euclea* exposure. By better understanding the impact of *Euclea* on broiler mortality rates, researchers can develop more effective and sustainable practices for poultry farming that prioritize animal welfare and productivity.

4. Conclusion

This study investigated the efficacy of using an aqueous leaf extract from the plant *Euclea Divinorum* to treat *Coccidiosis* (*Emeria tenella*) in chickens. The analysis of chemical compounds in the extract showed the presence of alkaloids, flavonoids, saponins, tannins, phenol, resin, glycosides and steroid rings. ANOVA analysis showed a significant difference in mortality among broilers treated with different levels of leaf solution. Broilers treated with T2 had the lowest mortality (10%), while broilers treated with T4 (66.7%) had the highest mortality. While *Euclea*, a natural plant extract, has shown promising results in treating *Coccidiosis*, there are several limitations to consider. One major limitation is the lack of standardized dosing guidelines and treatment protocols. Since *Euclea* is a natural product, its concentration and potency can vary depending on factors such as soil quality, climate conditions, and harvesting methods. This inconsistency makes it difficult to accurately determine the appropriate dosage for treating *Coccidiosis* in different animal species, leading to potential under-dosing or overdosing issues. Another limitation of using *Euclea* as a treatment for *Coccidiosis* is the lack of comprehensive research on its safety and efficacy. While some studies have shown positive outcomes, there is still a need for more rigorous clinical

trials and long-term studies to fully evaluate the effectiveness and potential side effects of this natural remedy. Additionally, the mechanism of action of *Euclea* in treating *Coccidiosis* is not well understood, which further complicates its use as a reliable treatment option. In conclusion, while *Euclea* shows promise as a natural alternative for treating *Coccidiosis*, its limitations in terms of dosing variability, lack of standardized protocols, and limited scientific evidence call for further research and caution when considering its use in clinical practice. Hence, the results show that the effectiveness of the plant extract does not depend on the concentration, and can be seen in the variations of broiler mortality in different treatment groups. In light of these findings, the following recommendations are proposed:

i. The promotion of large-scale cultivation of *Euclea divinorium* is recommended.
ii. Improved use of *Euclea divinorium* in the treatment of certain poultry diseases.
iii. Intensive research on plant extracts utilization in the treatment of poultry diseases.
iv. Adequate supply of subsidized vaccines and medicines to poultry farmers.
v. Improved extension services for farmers to control poultry diseases.

5. Acknowledgments

The study design and the first draft of the manuscript were written by the Authors. They were also responsible for processing the data, conducting the analysis, and searching the literature. The final manuscript was reviewed and approved by all the authors.

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