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Effect of the Aqueous Leaf Extract of Annona senegalensis in the Control of Coccidiosis of Birds

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Abstract

The study was carried out to evaluate the anticoccidial properties of aqueous leaf extract of Annona senegalensis. One hundred and twenty (120) broiler birds were after brooding for four (4) weeks experimentally infected with Eimeria tenella and divided into four groups T_1, T_2, T_3 and T_4 with three replicates in each group. They were subsequently treated with various concentrations of the aqueous leaf extract of Annona senegalensis at 1ml/ bird orally, twice a day for five (5) days. To T_1 was treated with 5mg/ml; T_2, 10mg/ml; T_3, 20mg/ml and T_4, 0mg/ml, respectively. Mortality rates of 33.3%(T_1); 6.7%(T_2); 0%(T_3) and 80%(T_4), respectively were observed. Duncan count was carried out on the dead and on some birds that survived for the scoring of hemorrhage. From the study, it is suggested that Annona senegalensis has great potentials in being used to control coccidiosis in birds given that T_3 (20mg/ml) had the lowest mortality rate (0%), while T_4 (0mg/ml) control had the highest mortality rate (80%). The phytochemical and chromatographic analysis of Annona senegalensis leaf showed the presence of Alkaloids, Saponins and Glycosides with Rf values values 0.63, 0.51 and 0.73 (not identified), respectively; Flavonoids chlorogenic acid, anthocyanin, rutin, catechol and quercetin with R_f values 0.75, 0.95, 0.22, 0.95 and 0.61, respectively; Tannin, catechin with R_f value 0.20; Terpenoids, kauronoic acid and oleanic acid with R_f values 0.39 and 0.51. Other active principles present include; Volatile oils and Steroids. From the ANOVA of the Duncan Count, results indicate that the degree of haemorrhage is concentration dependent.

Keywords: Coccidiosis; Eimeria Tenella; Annona Senegalensis; Phytochemistry; Thin Layer Chromatography

Introduction

Avian coccidiosis remains one of the most common diseases of poultry industry all over the world. It constitutes an important wet season hazard to poultry farmers in Nigeria [5]. Coccidiosis caused by Eimeria species cause huge economic losses in poultry and this includes the costs for treatment of birds, reduced productivity and losses due to mortality of birds. Since the introduction of various anticoccidial drugs and vaccines however, medications with these drugs have been fairly effective in preventing serious outbreaks of the disease; the reason for the emergence of resistant strains of coccidian parasites [5]. Besides the scarcity of livestock drugs, access to anticoccidial drugs which is difficult especially to the rural poultry farmers hence the urgent need to develop alternative drugs for the purpose of curing coccidiosis in poultry.

This necessitated the need to look into other ways of controlling these diseases notably the use of herbs or plants, such as Annona senegalensis which has been acclaimed both scientifically [15] and by traditionalists to have curative abilities for several diseases of both man and animals.

Annonia senegalensis is used traditionally as a stimulant, pain reliever and anti-snake venom. The plant has been reported to possess anti-oxidant, anti-microbial, antidiarrheal, anti-inflammatory, anti- parasitic, anticonvulsant, antimalarial and anti-nociceptive properties as well as many other biomedical properties of pharmaceutical relevance [14]. As per the traditional medicine practices, all the plant part of Annona senegalensis are useful in several diseases. The leaves have been used in treating yellow fever, tuberculosis and small pox [2]. The stem bark has been used in hernia treatment [8]. The root is used in conditions such as difficulty in swallowing, gastritis and male sexual impotence. The sap from the tree is used against venomous bites and in the management of diabetes. [13]. In guinea, Annona senegalensis, has been employed in the treatment of Malaria [17]. Among the Igede people of Benue State in North Central Nigeria, the plant is used in combination with Agaratum camycoides in the management of dysentery [11]. It has also been reported to possess antidrepanocytary (anti sickle cell anemia) and hypnotic activities.

Therefore, the use of this plant extracts as medicine may alleviates these difficulties, as they are not natural products but may comprise new therapeutic molecules to which resistance has not yet developed [1].

Methodology

Collection of Annona senegalensis Leaves

Fresh green leaves of Annona senegalensis was collected from the premises of Federal College of Forestry, Jos and was identified using stock number AH010 in the Herbarium unit of the College. The leaves were air dried at room temperature, ground into powder and stored in a cool dry place.

Preparation of Plant Extract

The aqueous leaf extract of Annona senegalensis was prepared by dissolving the dried pulverized plant in ethanol at the ratio of 1:3, left to stand for 24 hours at room temperature and filtered using Whatman's filter paper. The filtrate was then poured into stainless steel plates and dried in hot water bath. The dried extract of Annona senegalensis was then stored in a dessicator.

Preparation of Test Samples

Three (3) separate test samples were prepared by dissolving the dried extract of Annona senegalensis in distilled water (5mg/ml, 10mg/ml, and 20mg/ml), and stored in a refrigerator at 4^{o}C for 48 hours.

Phytochemical Screening of Powdered Plant Parts of Annona senegalensis

Phytochemicals such as Alkaloid, Tannin, Glycoside, Resin, Flavonoid and Saponin were analysed for based on standard acceptable scientific method [18].

Chromatographic Screening of Phytochemicals in Powdered Plant Parts of Annona senegalensis

The phytochemicals that were found present qualitatively were extracted and assayed chromatographically. The solvent system used was ethylacetate-methanol-water in the ratio of 100:13.5:10 [19].

Mineral Element Analysis

Various macro and micro elements were determined after ashing and digestion, using Atomic Absorption Spectrometer according to the method described by the Association of the Official Analytical Chemist [4].

Experimental Organisms

The experimental organism (Eimeria tenella) was sourced from the Parasitology Division of the National Veterinary Research Institute, Vom, Plateau State Nigeria.

Experimental Birds

One hundred and twenty (120) day-old broilers chicks were brooded for four (4) weeks within which routine management practices were observed. At three (3) weeks of age, the birds were divided into four (4) groups (T_1, T_2, T_3 and T_4) with three (3) replicates in each group.

Experimental Infection with Eimeria tenella and Treatment with the Plant Extract.

Challenge of birds with Eimeria tenella was carried out at 30 days of age for two consecutive days and on observation of clinical signs such as fever, ruffled feathers, anorexia, diarrhoea, bloody faeces, weight loss, emaciation [3], treatment with the plant extract commenced. To groups T_1, T_2 and T_3 were administered 5mg/ml, 10mg/ml and 20mg/ml of the aqueous leaf extract of Annona senegalensis at 1ml/ bird orally, twice a day for five (5) days, consecutively. To group T_4, no treatment was administered (control).

Performance Parameters.

The birds were observed daily for clinical signs. Morbidity and mortality rates were recorded daily in each sub-group. Necropsies were carried out on the birds that died during the experiment. Seven days after the challenge, the evaluation of caecal lesion was carried out on fifteen birds from each group (Duncan Count). A lesion score was assigned to from 0 to 4, where 0 corresponds to normal status with no gross lesion, 1 to small scattered petechiae, 2 to numerous petechiae, 3 to extensive petechiae and 4 to extensive haemorrhage that gives a dark colour to the caecal intestine [10].

Statistical Analysis

Data was subjected to analysis of variance (ANOVA) using statistical package for social science (SPSSLTD, working, surrey, UK). The significant treatment effect was discussed at probability significant means and was separated using the Duncan's new multiple range test and test was considered significant at a probability of $p<0.05$.

Result and Discussion

Annonia senegalensis is used traditionally as a stimulant, pain reliever and anti-snake venom. The plant has been reported to possess anti-oxidant, anti-microbial, antidiarrheal, anti-inflammatory, anti- parasitic, anticonvulsant, antimalarial and anti-nociceptive properties as well as many other biomedical properties of pharmaceutical relevance [14].

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Phytochemical Screening

The dried powdered leaves of Annona senegalensis was subjected to phytochemical screening for various phytochemicals according to the method [19]. Annona senegalensis leaf contains tannins, volatile oil, flavonoids highly present, alkaloids, glycosides, steroids and terpenoids moderately present, and saponins mildly present (Table 1).

Tannins is used in syptic preparations which produce contractions of blood vessels; stopping bleeding having the quality of retaining hemorrhages when applied to the bleeding part. Saponins have the property of causing haemolysis of cells even at low dilution, tends to be deposited on the surface of cells with which they come in contact and are not absorbed by the normal epithelium of the alimentary canal. The presence of saponin in the plant is a demonstration of the fact that the plant may have expectorant actions which are a very useful in the management of inflammation of the upper respiratory tract in addition to its cardio-tonic properties as reported by [9]. Alkaloid represents the active principle of vegetable drugs. They are alkaline in reaction and richly combine with acid forming salts soluble in water. All contain nitrogen. Some drugs may contain more than alkaloid and the actions of these may be antagonistic. Alkaloid

produces analgesic, anti- inflammatory and adaptogenic effects which help to develop resistance against disease and endurance against stress [10].

Glycosides have a tendency to block the conduction of the electrical impulse that causes contraction as it passes from the atria to the ventricles of the heart. Cardiac glycosides 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 pace marker region, the cells that rhythmically maintain the heartbeat [6].

Flavonoids is used as a supplement which reduces the symptoms of hemorrhoids. A number of flavonoids have been shown to have anti-inflammatory effects and to strengthen blood vessels. Flavonoids have been investigated for possible anti-inflammatory effects and anti-viral property. Chlorogenic acid is one of the most important flavonoids in the field of pharmacology- has been shown to have antibacterial, antimutagenic, antitumor, and antiviral activities, plus antioxidant and clastogenic activities. The transisomer acts as an insect oviposition stimulant, and it may also reduce larval growth [12].

Diverse functional roles of terpenoids have been critically studied and well-accepted now. Some of them include natural flavor additives for food or fragrances in perfumery and in traditional and alternate medicines as aromatherapy. Most comprehensively studied of which is the effect of terpenes in prevention and treatment of cancer. Illustratively, Taxol derivative (paclitaxel and docetaxel) are among the widely used drugs in cancer chemotherapy. Other important therapeutic uses of terpenoids include antimicrobial, antifungal, antiviral, antihyperglycemic, anti-inflammatory, antioxidants, antiparasitic, immunomodulatory, and as skin permeation enhancer [16].

Table 1: Phytochemistry of Annona Senegalensis leaves

Phytochemicals Tested	Observation
Alkaloids	++
Flavonoids	+++
Saponins	+
Tannins	+++
Terpenoids	++
Volatile oil	+++
Glycoside	++
Steroids	++

Key' – Negative, + Mildly Present, ++ Moderately present +++ Highly present

Chromatographic Screening of the Leaf of Annona senegalensis

Result after the chromatographic screening of the leaves of Annona senegalensis showed Alkaloids, saponins and glycosides with R_f values 0.63, 0.51 and 0.73 (not identified), respectively; Flavonoids chlorogenic acid, anthocyanin, rutin, catechol and quercetin with R_f values 0.75, 0.95, 0.22, 0.95 and 0.61, respectively; Tannin, catechin with R_f value 0.20; terpenoids, kauronoic acid and oleanic acid with R_f values 0.39 and 0.51, respectively (Table 2)

Table 2: Chromatogram of Active Principles Present in Annona senegalensis Leaf

Chemical Group	Rf Value	Possible Identity
Alkaloids	0.63	-
Saponins	0.51	-
Tannins	0.20	Catechin
Terpenoids	0.51 0.39	Kauronoic acid Oleanoic acid
Glycosides	0.73	-
Flavonoids	0.75	Chlorogenic acid
	0.95	Anthocyanin
	0.22 0.95 0.61	Rutin Catechol Quercetin

Mineral Element Analysis of Annona senegalensis leaf

Several minerals were observed in the leaf of Annona senegalensis; they include, calcium, copper, magnesium, manganese, phosphorus, potassium, Zinc and lead. It was observed that the concentrations of the minerals were exponentially increased in the dried Annona senegalensis leaf which may be as a result of the loss of moisture content in the dried leaves (Table 3).

Table 3: Minerals Present in Annona senegalensis leaf

Minerals	Fresh Leaves (mg)	Dried Leaves (mg)
Calcium	540	2185
Copper	0.9	0.62
Magnesium	56	602
Manganese	0.23	3.08
Phosphorus	115	453
Potassium	453	1676
Zinc	0.20	4.42
Lead	0.03	0.66

Statistical Analysis

From table 4, the null hypothesis is rejected and alternative hypothesis (Ha) accepted because the figure calculated is greater than the figure tabled from the ANOVA result. This indicates that there is a significant difference between the different levels of inclusion of aqueous leave extract of Annona senegalensis used in the experiment.

Performance of Birds infected with Eimeria tenella and treated with Annona senegalensis

Table 5, shows the mortality rate in percentage of birds infected with Eimeria tenella. In treatment four (T_4) which is the control had the highest mortality of 80% while treatment one (T_1) and two (T_2) has 33.3% and 6.7%, respectively and treatment three (T_3) had 0%; the lowest mortality rate. The result shows that the birds that were treated with 20mg/ml of the aqueous leaf extract of Annona senegalensis responded more positively to the treatment compared to other levels of concentration. Mortality result showed that a farm can have a 100% mortality rate when there is a coccidiosis outbreak.

Table 4: ANOVA of Degree of Hemorrhages of Birds Experimentally infected with Eimeria

Source of variance	Sum of square	Degree of freedom	Mean square SS/df	MST MSE	Critical value 5% TAB	1%
Total	143	11	13			
Treatment	29.6	3	9.86	0.69	4.07	7.59
Error	113.4	8	14. 18			

Source: Field Survey, 2020.

Table 5: Percentage mortality of Birds Experimentally targeted with different concentration of Annona senegelensis

Treatment	Number of Birds	Mortality Rate	(%)
T_1 (5mg/ml)	30	10	33.3
T_2 (10mg/ml)	30	2	6.7
T_3 (20mg/ml)	30	0	0
Control (0mg/ml)	30	24	80

Source: Field Survey, 2020.

Recommendations

Further research should focus on adequate dosage of the extract of the leaves of Annona senegalensis to be administered in the management of Coccidiosis and also the investigation of the plant for possible toxicity, even though there was no apparent negative observation made on the birds during the course of the research.

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