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# ANTICOCCIDIAL POTENTIALS OF *Cuccumis metuliferus* E. Mey. ex Naudin METHANOL EXTRACT IN EXPERIMENTAL BROILER CHICKENS

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## ABSTRACT

Coccidiosis is one of the most common and expensive diseases in the poultry industry. The disease is generally caused by a single celled parasite *Eimeriidae*. Anti-Coccidian potentials of *Cuccumis metuliferus* methanol extract was investigated in experimental broiler chicks with the intent of developing an affordable, accessible and available drug alternative. The whole plant was prepared and cold extracted using 70% methanol. Acute toxicity studies using set of five weeks old chicken grouped into six categories (A-F) was performed. To group (A) was administered distilled water as control, then, doses of 400, 800, 1200, 1600 and 2000mg/kgbw in increasing order were administered to birds in groups B-F. The birds reaction, body condition and number of mortality was monitored over 24 hours period in each group. Total numbers of 35 chicken was grouped into six (I-VI) with each group consisting of five (5) chicken. Fresh faecal from an infected bird containing *Eimeria* oocyst was collected. Compound Microscope was used to carry out the oocyst count and subsequent dilution to 74,000 oocyst/ml as an infective dose. The infection was carried out by administering 3ml of the infective dose to groups I-IV. The treatment with the extract at doses of 50, 100, 150 and 200mg/kgbw was given to groups I-IV while groups V and VI were the negative and standard controls respectively. Oocyst count was carried out using Neubear counter at 3 days interval. Doses of 400-1200 mg/kgbw did not cause any mortality while $LD_{50}$ was found at the highest dose of 2000mg/kgbw. The doses of 150 and 200 mg/kgbw were found to clear the Oocyst completely. Prophylaxis with 200mg/kgbw for five consecutive days prior to infection does not show any prophylactic effect. Result from this study reveals that methanol extract from *Cuccumis metuliferus* has pharmacological effect against *Eimeria* oocyst with a very low level of toxicity, though, no prophylactic effect.

**Keywords:** Coccidiosis, Poultry, Eimeria, *Cucumis metuliferus*, Parasites

## Introduction

Poultry industry is one of the rapidly growing major segments of agriculture sector and has been showing a tremendous growth during the last few decades. However, poultry production is often confronted by avian coccidiosis, flu, and other infectious diseases (Quiroz-Castaneda and Dant ˜ an-Gonz ´ alez, 2015). Coccidiosis is one of the most common and expensive diseases in the poultry industry (Shirley *et al.,* 2007). It has major economic impacts on poultry by reducing performance and decreasing productivity (Zhang, 2013). Coccidiosis is generally caused by a single celled-parasite in the sub kingdom Protozoa of the phylum Apicomplexa, the subclass *Coccidia*, family *Eimeriidae*. Chicken with coccidiosis are characterized by dysentery, enteritis, emaciation, drooping wings, poor growth and low production (Awais *et al.,* 2012). This disease is associated with high rate of mortality and morbidity (Shirzad *et al.,* 2011); this constitute a serious drawback and pose challenges for an industry that raises approximately 50 billion chickens annually, as a source of meat; accounting for over one-third of protein food

requirements for humans (Quiroz-Castaneda and Dant˜an-Gonzalez, 2015). Avian coccidiosis represents a serious disease that results in annual global economic losses of approximately $2.4-3.0 billion, including production losses and disease prevention and treatment costs (Blake and Tomley, 2014).

Current approaches to constrain avian coccidiosis include anticoccidial chemicals, vaccines, and natural products. Anticoccidial chemicals, coccidiocides, coccidiostats, and ionophores, have long been used as a mainstream strategy to control avian coccidiosis in modern poultry production (Chapman *et al.*, 2010). Although this strategy is cost-effective and successful, the presence of drug resistance and public demands for residue free meat has encouraged development of alternative control strategies (Chapman *et al.*, 2010). At least four plant products such as Cocci-Guard (DPI Global, USA,2016), a mixture of *Quercus infectoria*, *Rhus chinensis*, and *Terminalia chebula* (Kemin Industries, USA,2014), Apacox (GreenVet, Italy, 2016) and BP formulation made up of *Bidens pilosa* and other plants (Ta-Fong Inc., Taiwan)(Yang *et al.*, 2015) that are currently commercially available on the market which are used as anticoccidial feed additives in chickens and/or other animals. Besides, investigation of the compounds and/or their derivatives present in anticoccidial plants may inspire the research and development of anticoccidial chemicals. One successful example is halofuginone, a synthetic halogenated derivative of febrifugine, which was initially identified from the antimalarial plant, Chang Shan (*Dichroa febrifuga*) (Edgar and Flanagan, 1979).

The usefulness of plant materials in the treatment of diseases has been demonstrated to be as a result of the presence of certain chemical compounds in plants, which include flavonoids, alkaloids, steroids, tannins and saponins (Malik, *et al.*, 2014). Phytochemical compounds are non specific in their action and can exhibit several functions viz: antibacterial, anti-fungal, antiviral and anti-spasmodic (Sharma *et al.*, 2013). Such medicinal relief derived from plants phyto components include *Azadirachtin* in Neem leaves (National Research Council, 1993; Biu *et al.*, 2006) which has been reported to produce antiprotozoal, antibacterial and antifungal effects (Schmutterer, 1990). Other aqueous stem back extracts used for the treatment of coccidiosis include; *Khaya senegalensis, and Anona senegalensis*, *Aloe excels, Camellia sinensis, Curcuma longa, Echinacea purpure, Origanum vulgare, Saccharum officinarum, Triticum aestivum, Yucca schidigera* (Abbas *et al.*, 2012). Their photochemical analysis reveals that tannins, terpenes, anthraquinones, phlobitaminans, alkaloids, cardiac glycosides and steroids were present in various concentrations (Nwosu *et al.*, 2011).

*Cucumis metuliferus (*E. Mey. ex Naudin) belongs to the family Cucurbitaceae. It is commonly referred to as African horned cucumber, jelly melon, Kiwano in English. In Nigeria it is called ‘bùuràr zaàki’, ‘nòònòn-kuùraà’ ‘gautar kaji’ by the Hausas (Burkill, 1985). It is a monoecious, climbing and annual herb that can be grown practically anywhere provided the season is warm (Benzioni *et al.*, 1993). The fruits are ovoid berries of 8-10 cm long and 4-5 cm in diameter, reddish orange at maturity covered with strong spiny outgrowths.

In the current study, the efficacy of the methanol extract of *Cucumis metuliferus (*whole plant) against experimental Coccidiosis was evaluated.

## Materials and Methods

### Experimental site

The research work was conducted at both the teaching/research farm and the central laboratory of the Federal College of Wildlife Management, New-Bussa, Niger State. Middle belt region of Nigeria. The experimental station (New Bussa) sits at 9°53'N ,9.883°N and 4°31'E, 4.517°E (NIPOST Archives, 2009). The research work was carried between the Months of May to July (early part of rainy season)

### Experimental animals

Day old Cornish Cross Broilers broiler chicks were purchased from Ibadan Oyo State. They were kept in the research pen and fed with broilers feed and with clean water made available ad-libitum. High standard of health/sanitary conditions was maintained throughout the course of the research work.

### Plant materials

Ethno-botanical survey was carried out in the surrounding villages old/new Awuru, Koro, Popo, Kere, Lubaruru and Dogongari villages with the main aim of ascertaining from the local people (particularly the elderly ones), the plant species(s) commonly utilised in the traditional management of coccidiosis. Part(s) used, method of preparation and period of harvest was enquired from the interviewees'.

### Plant preparation:

The whole plant (*Cuccumis metuliferus*) was washed with clean water and dried at room temperature and then chopped into smaller fragments and pulverised using a grinder. Cold extraction was performed using 70% methanol which was filtered after 24hrs and then dried using steam bath. The extract was transferred into the sample bottle and stored in the refrigerator at $4^{O}$C until required for use.

### Experimental Drugs

Amprolium (Amprolium 200 NTCOX 20%,) a commercially available anticoccidial drug for the routine treatment of an avian coccidiosis (due to *eimeria* spp) in Nigeria was used to compare the anticoccidial effects of the plant extracts.

### Phytochemical Analysis of the Methanol Extract of The Plant

The whole plant extract was analysed for phytochemical constituents including tannins, saponins, alkaloids, anthraquinones derivatives, terpens, steroids and cardiac glycosides as described by Trease and Evans (2002).

### Pharmacological Studies

### Acute toxicity studies

Twenty four (24), finisher Cornish Cross Broiler chickens were grouped into six (A-F) of four (4) chickens each. To group A, was administered distilled water (as control), then, extract at doses of 400, 800, 1200, 1600 and 2000mg/kgbw in increasing order was administered to groups B-F. The bird's reaction, body condition and number of mortality was thereafter, monitored over 24 hours period in each group.

### Harvesting of the infective parasites samples

Simple random sampling method was used to collect feacal samples from poultry dressing slabs in New Bussa, Monday Market, Sabon-Pegi market and Wawa markets as well as from the FCWM research poultry farms. Pooled fecal samples were aseptically collected from bird's fresh droppings in poultry farms or cutting open freshly eviscerated intestine of slaughtered chickens, squeezing out the feces into a sterile labeled polythene bags and immediately transported to the Veterinary Clinic dressing room, Department of

Animal Health and Production Technology, FCWM for further parasitological analysis.

**Isolation of the *Eimeria oocyst***

The fecal samples were soaked overnight at 37°C in 2.5% (w/v) aqueous solution of potassium dichromate. The samples were shaken vigorously to break up the feces. The suspension was filtered through cheesecloth into a beaker. The filtrate obtained was centrifuged at 447 ×*g* (Rotor radius = 10) for 5 min to settle down the oocysts. The supernatant fluid was discarded and the *Eimeria* oocysts present in the sediment were separated using floatation technique and then examined carefully through microscope using oil emersion lens for the presence of the *Eimeria* oocysts. Counting of oocysts was done using McMaster counting technique and was expressed as per gram of feces (Lawal *et al.*, 2016). This was subsequently diluted to the standard infective dose of 74,000 oocysy/ml as described by Conway and McKenzie (2007).

**Anti-coccidial Effect**

Total number of 35 Finisher (Cornish Cross Broiler) chickens were grouped into six (I-VI) with each group consisting of 5 chickens as thus:

Group 1 = 50mg/kgbw
Group 11 = 100mg/kgbw
Group 111 = 150mg/kgbw
Group IV = 200mg/kgbw
Group V = infected and not treated
Group VI = treated with the standard drug

**Infection of Animals**

The infective dose of the culture (that is, 74,000 oocyst/ml) was administered orally using appropriate syringe to groups I-VI. Thereafter, treatment was carried out using the extract on groups I-IV on two days interval for 21 days (3 weeks). Group V was neither administered the extract nor the standard drug (negative control) while to group VI, the standard drug was administered.

**Microscopic examination of fecal samples**

This was carried out based on previously described methods of Lawal *et al.* (2016) and Conway and McKenzie (2007) (Refer to the **Isolation of the *Eimeria oocyst*** section) The count was at 3 days interval.

**Prophylactic activity test**

The highest effective dose of 200mg/kgbw was administered to a group of five chickens for seven consecutive days. Thereafter, they were infected as described previously. Oocyst count was carried out at three days interval also as previously described.

**Statistical analysis**

The data collected were subjected to one way analysis of variance (ANOVA) in a completely randomized design (CRD) arrangement. The significant means were separated and compared using Duncan multiple range test (DMRT).

**Results and Discussion**

Plants produce a broad-spectrum variety of phytochemicals such as phenolics, polyacetylenes, alkaloids, polysaccharides, terpenoids, and essential oils with a large number of bioactivities (Bozkurt *et al.*, 2013).

**Table 1. Phytochemical constituents of *Cucumis metuliferus methanol* extract.**

| Constituents | Aqueous extract | Methanolic extract |
|---|---|---|
| **Alkaloids** | +++ | ++ |
| **Tannins** | ++ | ++ |
| **Flavonoids** | +++ | ++ |

| | | |
|---|---|---|
| **Carbohydrate** | ++ | +++ |
| **Steroids** | — | — |
| **Anthraquinones** | — | + |
| **Saponins** | + | ++ |
| **Cardiac glycosides** | ++ | ++ |

**+ =Trace amount ++= Moderate amount +++= Large amount - = Absent**

As revealed in table 1, phytochemical analysis of the extract showed the presence of these compounds except the steroids and this agrees with the findings of Nwadiaro *et al.* (2015). The alkaloids in the fruit have been found to be much more than that of the leaves as reported by Aliero and Gumi (2012); Usman *et al.* (2014). Therefore, the high concentration of the alkaloids observed in this study is a result of using the whole plant parts. Hassan *et al.* (2004) reported that these classes of compounds (alkaloids, saponins, tannins, anthraquinones, and flavonoids) are known to have curative activity against several pathogens and therefore could suggest the use locally for the treatment of various illnesses. Due to their ability to modify the body's reaction to allergies, viruses and carcinogens, flavonoids have been referred to as nature's biological response modifiers (Nwadiaro *et al.*, 2015). The flavonoids from *C. metuliferus* have demonstrated antiviral properties (Wannang, 2010). Tannins also decrease bacterial cell proliferation by blocking key enzymes of microbial metabolism (Awosika, 1991). Flavonoids, tannins and saponins were also reported to have inhibitory effect on the growth of *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Candida albicans* thereby supporting the antimicrobial potential of the plant (Manthey, 2000). The bioactive principles have also been known as the defensive mechanism of the plants against different pathogens (WHO, 1998).

Preliminary oral administration of the extract to groups of birds at increasing doses with a record of minimum mortality is a clear indication of the non-acute toxicity of the extract from *Cucumis metuliferus*. The $LD_{50}$ was determined at the dosage of 2000 mg/kgbw (Table 2).

**Table 2. Acute toxicity of Cuccumis *metuliferus* methanol extract**

| Dosage | No of Animals | T/D | Observations |
|---|---|---|---|
| Distilled $H_20$ or Normal Saline | 4 | 4/0 | No sign of toxicity, animals remained active even after the administration. |
| 400mgkg-1bw | 4 | 4/0 | No sign of toxicity, animals remained active even after the administration. |
| 800 mgkg-1bw | 4 | 4/0 | Looked a bit depressed, the breathing was slow and remained Sluggish for a short while became normal again. |
| 1200 mgkg-1bw | 4 | 4/0 | Sluggishness was observed, the breathing was slow and there was closing of the eyes and the feathers stood erect but conditions returned to |

| | | | |
|---|---|---|---|
| | | | normal after about 24h. |
| 1600 mgkg-1bw | 4 | 4/1 | One death was recorded about 13 h after the administration of the fraction and it took almost 27h before the animals recovered fully from the sluggishness, depressed breathing, and erected feather. |
| 2000 mgkg-1bw | 4 | 4/2 | Two deaths were recorded about 17 h after administration of the extract and it took almost 48h before the animals recovered fully from the sluggishness, depressed breathing, erect fur and closing of the eyes. |

**T/D = Ratio of number of death recorded from the total number of birds**

Administration of various doses of *C.metuliferus* extract to the experimental chickens shows Anticoccidial activity in a dose dependent manner and compete favourably with the standard drug (Amprolium). When 50mg/kgbw was administered, the fecal oocyst count seems to fall more drastically compared to the standard drug on the fourth day but the count thereafter, remains higher than that of the standard drug in the subsequent days. While the complete clearance of the parasites was attained on the 24$^{th}$ day into the treatment with the standard drug (Amprolium), very low fecal oocyst counts of 0.2 and 0.1x $10^6$/g was attained with the doses of 150 and 200mg/kgbw of the crude extract respectively. (Fig. 1). This result when compared to the findings of Loredana *et al.* (2019) in their application of herbal formula to treat coccidiosis and Mzena *et al.* (2018) in their study of Antimalaria activity of *Cucumis metuliferus* and *Lippia kituiensis* against *plasmodium berghei* infection in mice, follow similar trend.

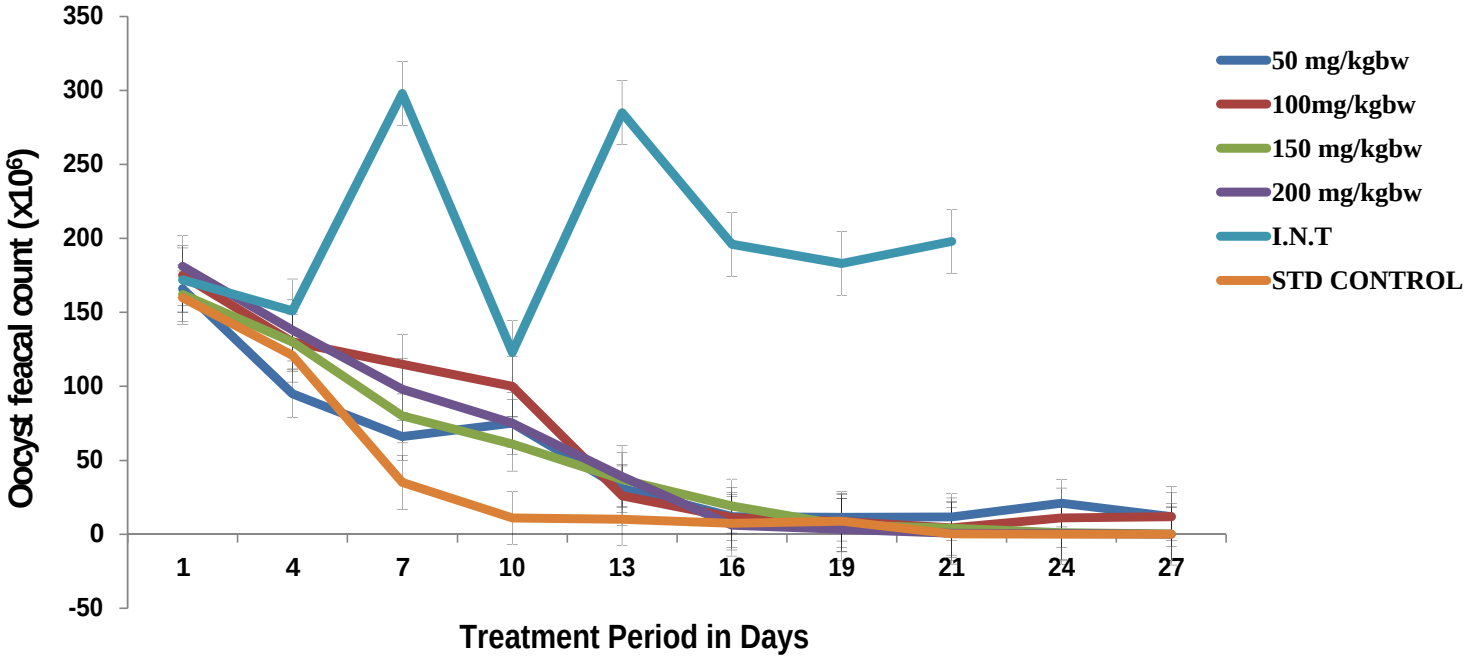
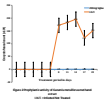



**Figure 1 Anticoccidial effect of variuos doses of Cucumis metuliferus extract on experimental rats**
**I.N.T.==Infected Not Treated STD == Standard**

The prophylactic potency of the extract was found to be encouraging due to the fact that, the highest dose of 200 mg/kgbw administered to the group of five chickens for five consecutive days prior to their infection suppressed the parasites, in the first three days, but thereafter, came up, though at much lower count when compared to the negative control (Figure 2). This finding conform with the results reported by Lydia,(2018); Joy *et al.* (2018); Alli *et al.* (2011)



Worthy of note is the presence of carbohydrate (as a component of phytochemicals) in large quantity. Some of this carbohydrate in *C. metuliferus* were found to consist of some oligosaccharides such as inulin, arabinoxylooligosaccharides (AOS), fructooligosaccharides (FOS), mannanoligosaccharides (MOS),

xylooligosaccharides (XOS), isomaltooligosaccharides (IMOS), soy oligosaccharides (SOS), and pyrodextrins (Al-Sheraji, *et al.*, 2013). These oligosaccharides were reported to possess some prebiotic properties as a result of being non-digestible feed ingredients that promote the growth of probiotics and their activities in guts (Bindels *et al.*, 2015). The mechanism of these oligosaccharides is through selective stimulation of beneficial bacteria in the intestinal system of the bird. The increasing number of beneficial microbiota excludes the harmful pathogens from colonization in the intestinal track of the bird. Subsequently, healthy hosts can produce a wide variety of bacterions and other immuno-modulators that can stimulate macrophages to neutralize the pathogens (Alloui *et al.*, 2013). Moreover, Bozkurt *et al.* (2014) reported that prebiotics diminished coccidial infection in chickens but kept marginal oocyst production that might serve as a source of live vaccine for uninfected chickens.

## Conclusion

Despite the fact that, this disease is controlled mainly by hygiene and use of chemical anti-coccidial agents. However, cost and indiscriminate use of anti-coccidial drugs including Amprolium, monensin, ionospheres and nicarbazin have resulted in the emergence of resistant strains of *Eimeria*. These have cut short the usefulness of successive commercial drugs thereby prompting the exploration of new areas for appropriate alternative control strategies that are more affordable, accessible and readily available to both the large intensive and rural small holder poultry farmers. Results obtained from this study revealed with some degree of confidence that *C. metuliferus* could be a cheaper and locally available alternative.

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