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# In-Vitro Anticoccidial Efficacy of Crude Methanol Extracts of Selected Medicinal Plants against Eimeria Tenella

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## Abstract

A study was carried out to evaluate in-vitro anticoccidial efficacy of methanol extracts of Azadirachta indica, Vernonia amygdalina, Nicotiana tabacum, Moringa oleifera, Croton macrostachyus, and Hagenia abyssinica against Eimeria tenella. None-sporulated oocysts of Eimeria tenella isolated from experimentally infected chickens and exposed to crude extracts concentration of 100mg/ml, 50mg/ml, and 25mg/ml. Amprollium 1.5 mg mL-1 and 2.5% K2Cr2O7 used as positive and negative control, respectively. The result showed dose and time dependent sporulation inhibition, was seen in oocysts exposed to crude extracts of Vernonia amygdalina, Croton macrostachyus and Moringa oleifera. The better efficacy was seen with Moringa oleifera. The higher and lower percentage of sporulated oocyst was (0%, 5%), (0%, 0.5%) in Vernonia amygdalina and Moringa oleifera, respectively. The study also showed that the exposure to higher concentration of crude extract (100mg/ml) produced greater proportion of oocyst wall distortion which was 6,000, 9,600 and 5,400 for Vernonia amygdalina, Croton macrostachyus and Azadirachta indica, respectively. Phytochemical analysis of this plants showed the presence of bioactive metabolites such as saponins, tannins, alkaloids, steroids and glycosides and flavonoids, which may contributes for in vitro anticoccidal effects. These results are of a major importance as contamination of poultry houses with coccidian oocysts is very difficult to control and all attempts to eradicate infections with Eimeria spp. Therefore further studies aimed to confirm the pharmacological effects of these plants, toxicity evaluation and in vivo studies need to be done before the usage of these plants.

**Keywords:** *Eimeria • In vitro • Medicinal Plants • Poultry*

## Introduction

In East Africa, over 80% of human population lives in rural areas and over 75% of these households keep indigenous chickens [1-2]. Ethiopia has large population of chickens estimated to be 48.9 million with native and exotic chickens breed [3]. Though chicken production widespread, it faced with wide range of problems including disease [4]. Coccidiosis is ubiquitous in intensive production system, where it causes greatest economic impact due to production losses and costs associated to control and prevention [5]. The most common conditions that facilitate the development of disease includes the type of Eimeria species, number oocyst, poor ventilation, high humidity in litter, high stocking density, low immune status of the host, bacterial enteritis, and lack of effective anticoccidial drugs. Chickens are commonly susceptible to different species of Eimeria including Eimeria tenella, Eimeria maxima and Eimeria acervulina, of these Eimeria tenella causing the caecal coccidiosis is highly pathogenic. The pathological effect of Eimeria is associated with replication of Eimeria sporozoites in the epithelial cells of the intestinal tract, which leads to tissue damage that cause poor nutrient absorption, dehydration and blood loss. Coccidiosis is controlled by the use of anticoccidial drugs. But the development of resistance to available anticoccidial drugs by different Eimeria species is current challenge.

Plants contain different biomolecules with diver's pharmaceutical values. Herbal plants of Vernonia amygdalina (VA), Azadirachta indica (AI), Tobacco and Moringa have been considered as, chemo-preventive and chemotherapeutic agents against breast cancer, antibiotic, fungicidal, anthelmintic, enhanced immune response of poultry and animal, therapeutic immune-modulatory molecules, like cytokines. Studies demonstrated on the potential benefits of phytochemicals such as saponins, nicotin, tannins, alkaloids, flavonoids, terpenoids and phenolic compounds against avian coccidiosis [16]. An in vitro experiment showed sporulation inhibition effects of herbal extracts against coccidian oocysts. In recent years,

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there has been increasing global interest in ethno-veterinary practices in treating various ailments. The increased interest of animal production free from industrial chemical inputs and drug resistance issues, are among pushing factor to search new therapeutic substances of natural origin, with possibly low toxicity to man and animals. Moreover, accessibility of different medicinal plants creates opportunity for traditional medicine. However, in Ethiopia anticoccidial efficacy of medicinal plants used in the treatment of chicken coccidiosis have not been scientifically evaluated and therefore not fully known [6]. Therefore, the study was done to investigate the in vitro effect of crude extract of selective medicine plant against pathogenic Eimeria species (Eimeria tenella).

## 2. Materials and Methods

### Study area description:

The study was conducted from September 2018 to March 25, 2019 in Haramaya University, Ethiopia. The area is approximately 20 km from west of Harar and 510 km east from Addis Ababa. The estimated animal population in the area is about 63,723 cattle, 79,950 sheep, 120,350 goats, 30,280 donkeys, 480 camels and 120,235 chickens. Topographically, it is situated at altitude of 1600 to 2100 m above sea level with the mean annual temperature and relative humidity of 18°C and 65%, respectively. Geographically it is located 041° 59' 58" N latitude and 09° 24' 10"S longitudes. There are four seasons; a short rain season a short dry season, a long wet season and a long dry season.

### Plant Preparation:

The fresh leave of Azadirachta indica, Vernonia amygdalina, Nicotiana tabacum, Moringa oleifera, Croton macrostachyus, and Hagenia abyssinica were collected from different parts of east, Ethiopia, under the supervision of botanists. Plants specimen was identified and voucher specimen was deposited at the School of Plant Sciences, Haramaya University. Approximately 1kg fresh leaf were separated from the plants and washed thoroughly for 2-3 times with running clean tap water and followed shade drying. During plants collection aseptic techniques followed to reduce possible contamination. The plants leaves, spread out on paper sheets, dried in shaded area at room temperature for two weeks and finely powdered with the help of grinder. A 25-mesh diameter sieve was used to obtain fine powder and then preserved for extraction. One to five ratio (1:5) of leaf dry powder to solvent, soaked in methanol and shake for 24h in shaker 80 rpm at 370c [7]. The mixture was later strained using a muslin cloth and filtered using a Whitman® filter paper (No. 1) and the filtrate was concentrated in a vacuum rotary evaporator at 400c and evaporated to dryness at 37°C in air oven. After complete solvent evaporation, the filtrates were stored in capped labeled bottles and kept in the refrigerator at 4°C until use [8].

### Phytochemical Screening:

The crude methanol extract of plants was subjected to phytochemical screening using a standard screening procedure for the presence of secondary metabolites such as: saponins, tannins, phenolics, alkaloids, steroids, flavonoids, glycosides and phlobatannins using the method described by Harborne, [9].

### Working Concentrations:

The stock solutions of the crude extract obtained from test plants were prepared by dissolving the crude extract in distil water. For in vitro screening, working concentration of 100, 50, 25 mg ml-1 of crude extract was prepared in addition, amprollium 1.5 mg mL-1 and 2.5% K2Cr2O7 used as control group.

## 3. Results

Crude extract of each plants (Azadirachta indica, Vernonia amygdalina, Nicotiana tabacum, Moringa oleifera, Croton macrostachyus, and Hagenia abyssinica) contain secondary metabolites such as: saponins, tannins, alkaloids, steroids and glycosides and flavonoids in almost all plants except some difference in the degree of color changes which may show the difference in the quantity. Sporulation inhibition test on Eimeria tenella oocyst showed, for all the concentration of Vernonia amygdalina, Croton macrostachyus and Moringa oleifera, there was an increase in sporulation inhibition with increasing concentration of the extracts and duration of exposure. The high efficacy was seen with Moringa oleifera at 24hr, 48hr and 96hr for all the concentration. The high and low percentage of sporulated oocyst was (0%, 5%), (0%, 0.5%) for Vernonia amygdalina and Moringa oleifera, respectively. Regarding oocyst exposed to different concentration of Hagenia abyssinica, Nicotiana tabacum, Croton macrostachyus and Azadirachta indica, it was seen low degree of inhibition. Amprollium, produce comparable efficacy with Vernonia amygdalina, and Moringa oleifera with 2.6%, sporulated oocyst.

## 4. Discussion

Oocysts wall of Eimeria made up of tyrosine rich cross-linking proteins which responsible for the notorious resilience of oocysts [10]. Recently, several in vitro experiments have proved anticoccidial effects of different herbal extracts and essential oils for the control of coccidiosis in birds [11]. Phytochemical analysis of plants showed the presence of bioactive metabolites such as: saponins, tannins, alkaloids, steroids, glycosides and flavonoids. Researches showed tannins, flavonoids and saponins known to have anticoccidial activity by decreasing sporulation. In the present study extracts from Vernonia amygdalina, Croton macrostachyus, Moringa oleifera and Azadirachta indica showed better in vitro anticcocidal effects. This may be associated with the higher quantity of secondary metabolites.

## 5. Conclusion

The present study reported that Vernonia amygdalina, Moringa oleifera, Azadirachta indica and Croton macrostachyus possessed promising in vitro anticoccidial activity and could be useful as alternative product for the control of avian coccidiosis in poultry production in future. Especially high dose of crude extract and long exposure time produce better anticoccidal effects. Extracts of Hagenia abyssinica and Nicotiana tabacum had low efficacy on the Eimeria tenella. These results are of a major importance as

contamination of poultry houses with coccidian oocysts is very difficult to control and all attempts to eradicate infections with Eimeria spp. Further studies aimed to confirm the pharmacological effects of this plant, toxicity evaluation and in vivo studies need to be done before the usage of the plants.

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## Competing Interests

The authors declare that they have no competing interests.

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