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Comparative Study of In-Vitro Anti-Coccidial Efficacy of *Allium Sativum* and *Carica Papaya*

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ABSTRACT

A comparative study was carried out to evaluate the in-vitro anticoccidial efficacy of aqueous and powder extract of A. sativum and C. papaya. A total of 4800 unsporulated but viable oocysts of Eimeria species isolated from naturally infected chickens were exposed to dry crude extracts powder concentration of 2.5g, 5g and 10g and aqueous concentration of 2.5g, 5g and 10ml/litre of water of Allium sativum and Carica papaya extracts in distilled water (g/L) and (ml) respectively. Oocysts were examined under phase contrast miscroscope and number of sporulated oocysts from the earlier unsporulated oocysts exposed to each concentration was counted at 1hr, 18hrs and 48hrs interval. The highest efficacy at 1hr, 18hr and 48hr for all the 3 preparations were obtained when 4800 oocysts were exposed to 10g of either extracts as against 2.5g and 5.0g. Amprolium achieved highest efficacy at 1hr, 18hr and 48hrs of exposure with 74%, 84% and 91% unsporulated oocyst respectively when given at recommended dose of 1.5g in drinking water. Amprolium was found to be more effective in the treatment of coccidiosis than Allium sativum and Carica papaya extracts. On the other hand, A. sativum exhibits anticoccidial activity than C. papaya although the difference in efficacy of the two extracts was not statistically significant ($p>0.05$). However, it was experimentally proven from the invitro study that any of the used extracts may be alternatively use for the control of coccidiosis where orthodox drugs such as Amprolium are not available.

Keywords: *Allium sativum, Carica papaya, Amprolium, Efficacy*

INTRODUCTION

Coccidiosis is a parasitic disease of the intestinal tract of animals caused by single-cell protozoa of the genus Eimeria or Isospora. Infection in poultry usually results in enormous economic losses as a result of the associated morbidity and mortality (Oluyemi and Roberts, 2000). Coccidiosis in birds like in other animals usually leads to extensive destruction of the intestinal epithelium resulting in reduced food efficiency and body weight gains, temporary reduction in egg production and sometimes death (Dalloul and Lillehoj, 2005). In poultry, three species of Eimeria have been reported to affect poultry birds in different part of the world. These include *Eimeria tenella*, *Eimeria maxima*, and *Eimeria acervulina* (Shirley, 1995; El-Khta *et* al., 2014).

Infection in birds is contracted through the ingestion of hardy, thick-walled sporulate doocysts which are able to survive for lengthy periods in poultry litter and in the soil. In other words, coccidiosis is commonly associated with poor hygienic measures and poor use of chemotherapeutic agents or chemical anticoccidial agents (Soulsby, 2002). Although there has been a rapid growth of the poultry industry over the past few decades, the success is dependent on the effective use of anticoccidial drugs, improved hygiene and availability of clean water among others. However, the emergence of drug resistance and the escalating cost of coccidiocidal drugs are now becoming major constraints in coccidiosis control in most livestock farms including poultry. In addition, there is increasing demand for poultry and poultry products that are free from residual anti parasitic drugs in recent years (El-Khtam *et al*., 2014). These among other reasons couple with drug resistance have raised the need for alternative treatment of poultry diseases including coccidiosis. Plants such as *Aloe vera, Allium sativum*, *Carica papaya* among others have been reported to contain natural antimicrobial properties (Iciek *et al*., 2009).

Phytochemical compounds of *Allium sativum* (garlic) have been shown to inhibit the viability of certain micro-organisms such as bacteria, protozoans and fungi. Several bioactive compounds have been identified in garlic. Iciek *et al*. (2009) and Lanzotti (2006) reported the

presence of Allicin (allyl 2-propenethiosulfinate or diallylthiosulfinate) in aqueous extract of garlic homogenate. Other important compounds reported in garlic homogenate include 1 - propenylallylthiosulfonate, allyl methyl thiosulfonate, (E, Z)-4,5,9-trithiadodeca- l,6,11-triene 9- oxide (ajoene), and y-Lglutamyl-S-alkyl- L-cysteine. These bioactive compounds are reported to induce oxidative stress and also neutralize reactive oxygen species leading to death of the parasite (Lanzotti, 2006).

Carica papaya Linn (Paw-paw) on the other hand has been reported to possess excellent medicinal properties for the treatment of different ailments. Different parts of the *Carica papaya* plant (leaves, seeds, latex and fruit) have been used for its medicinal value. Latex from unripe papaya fruit contains the enzymes papain and chymopapain; and a mixture of cysteine endopeptidases, chitinases and an inhibitor of serine protease (Kirtikar andBasu, 1998). Phytochemical analysis of *C. papaya* leaf extract revealed the presence of alkaloids, glycosides, flavanoids, saponins, tannins, phenols and steroids which could be useful in the treatment of different diseases including coccidiosis (Kirtikarand Basu, 1998). Information on the efficacy of these plants against coccidiosis in poultry birds would significantly enhance control of coccidiosis in poultry. In addition, garlic and papaya are grown widely in Nigeria and extracts derived from these plants have been reported to be safe and environmentally friendly. Hence a study is design to find alternative strategies to synthetic coccidiostats for treatment and control of coccidiosis in chicken using these two medicinal plants (*Allium sativum* and *Carica papaya*). The objective of this study was to evaluate the efficacy of *Allium sativum* and *Carica papaya* aqueous and powders against through in vitro experimental design.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh *Allium sativum* Linn and unripe fruits of *Carica papaya* Linn were bought from an urban market (Chechenia) in Kaduna, Kaduna State. Plant samples were transported in polythene bags to the Department of Biological Sciences Laboratory, Nigeria Defence Academy for identification. In the laboratory, the bulb and fruit of sampled plants were identified by a Botanist and assigned a voucher number.

Preparation of Plant Extracts.

Aqueous and dehydrated extract of garlic extract and pawpaw extract were prepared according to the method of Parekh and Chanda (2006).

Preparation of Aqueous Plant Extracts.

A.sativum (Garlic)

Aqueous garlic extract was prepared according to the method of Parekh and Chanda (2006). Briefly, extracts was prepared from fresh 500g garlic bulb by crushing surface-sterilized cloves with a ceramic mortar and pestle. The pulp was then suspended in 1000ml of sterile distilled water, filtered through sterile cheese cloth into a basin, and the resulting "stock" solution was diluted at 5 and 10ml/L of distilled water to obtain final garlic concentrations.

C.papaya (Pawpaw)

Aqueous extraction was also carried out according to the method of Parekh and Chanda (2006) using the seeds of unripe pawpaw. Papaya seeds weighing 500g were grinded with the aid of a ceramic mortar and pestle. The grinded powder was then suspended in 1000ml of sterile distilled water and then filtered through a sterile cheese cloth into a flat bottom flask to form a stock solution. The resulting stocks solution was diluted at 5 and 10ml/L of distilled water to obtain final papaya concentrations.

Preparation of Dehydrated Plant Extracts.

A. sativum(Garlic)

Dehydrated *A. sativum* extract was prepared according to the method described by Parekh and Chanda (2006). The unpeeled garlic cloves weighing 500g were cut into pieces and sun dried for 3 days using a flat stainless tray. Dried pieces of garlic cloves were then ground in a wooden mortar and pestle into powder and stored in vacuum-sealed polyethylene bags at room temperature until needed.

C. papaya (Pawpaw)

Seeds of unripe *C. papaya* fruit were removed and spread in a big stainless tray. They were subsequently kept under the sun regularly for 3 days for it to be dehydrated. The dried seeds weighing 500g were then grinded using a wooden mortar and pestle to obtain the powder form which was stored in vacuum-sealed bags at room temperature as described by Parekh and Chanda (2006).

Experimental drug

Commercial available anticoccidial drug (Amprolium) for the treatment of coccidiosis was used to compare the anticoccidial efficacy of the aqueous and powder extract of *A. sativum* and *C.papaya*.

Collection and Isolation of Eimeria tenella Oocyst

Collection of faecal samples from naturally infected birds

Fresh faecal samples from chickens naturally infected with Emeria species were collected from the college of Agriculture poultry farm Mando, Kaduna, Kaduna State, Nigeria, using a wide mouth sample bottle. Samples collected were placed on ice in thermox box and transported to the Department of Biological Sciences Laboratory NDA Kaduna. In the Laboratory, faecal sample was transferred to a 50ml test tube containing 2% Pottssium Dichromate.

Isolation of Oocyst

Oocysts were isolated using sieving and sedimentation techniques as described by Soulby (2002). Briefly, samples were mixed with 9.5% physiological saline, sieve in a 150 micro-mesh to remove debris and the filtrate transferred to centrifuge tubes and centrifuged at 1,500 rpm for 5 min. Heavy material in the droppings settled at the bottom while the supernatant was decanted. The sediment in the centrifuged tubes was then mixed with 15ml Aluminium Nitrate and kept in the refrigerator until needed.

In-vitro Sporulation inhibition assay

Purified oocysts (4800) of *E. tenella* were exposed to various concentrations of garlic and pawpaw extracts at w/v 2.5, 5.0, and 10g/distiled water and v/v; 2.5, 5.0, and 10ml/distilled water respectively, and Amprolium at manufacturer's recommended concentration of 1.25g/liter to serve as reference drug. Three replications were made for each concentration and the control.

Petri dishes in triplates containing either garlic or papaya extract at different concentrations of w/v 2.5, 5.0 and 10g/distilled water were inoculated each with 4800 unsporulated but viable oocysts and incubated at room temperature in the laboratory. Oocysts were examined under phase contrast miscroscope and number of sporulated oocysts from the earlier unsporulated oocysts exposed to each concentration was counted at 1hr, 18hrs and 48hrs interval. Oocysts with 4 sporocysts were considered sporulate dregard less of the shape and size of the sporocysts.

Data analysis

Data generated from the study was analyzed using Statistical Package for Social Scientist (SPSS) version II (2011). Analysis of variance was used to determine significance between groups, simple percentage was used to determine percentage reduction while least significant difference (LSD) was calculated at $p \leq 0.05$.

RESULTS

For all the 3 preparations (Aq. extracts of *A. sativum*, Aq. extracts of *C. papaya* and Aq. extracts of *A.sativum*+Aq. extracts of *C. papaya*), there was an increase in efficacy with increasing concentration of the extracts and the duration of exposure of oocysts to the extracts. The highest efficacy at 1hr, 18hr and 48hr for all the 3 preparations were obtained when 4800 oocysts were exposed to 10g of either extracts as against 2.5g and 5.0g. The percentage of unsporulated oocyst achieved with the 3 preparations ranges between 54% and 79% (Table 4.1). Although highest number of unsporulated oocyst was obtained with 10g of all extracts tested, an efficacy range of 49% - 68% was obtained with the 3 preparations when oocyst were exposed to either extracts at 5g concentration. However, the efficacy of *A. sativum* and *C. papaya* when used in synergy was generally higher than *C. papaya* when used singly, *A. sativum* alone achieved higher efficacy than when in synergy with *C. papaya* at 2.5g, 5g and 10g concentration. Amprolium, a synthetic coccidiostat achieved highest efficacy than all the preparations at 1hr, 18hr and 48hrs of exposure with 74%, 84% and 91% unsporulated oocyst respectively when given at recommended dose of 1.5g in drinking water. Statistical analysis shows that, though there are variations in the efficacy of the preparations, the difference was not statistically significant at a given concentration, ($P \leq 0.05$) however there was a significant difference when compared with Amprolium ($P \leq 0.05$)

Efficacy of crude powder extracts of *A. sativum* and *Carica papaya* on the viability of coccidian oocyst in-vitro. For all the 3 preparations (*A. sativum, C. papaya* and *A. sativum + C. papaya* powder), there was an increase in efficacy with increasing concentration of the powder extracts and the duration of exposure of oocysts to the extracts. The highest efficacy at 1hr, 18hr and 48hr for all the 3 preparations were obtained

when 4800 oocysts were exposed to 10g of either extracts as against 2.5g and 5.0g. The percentage of unsporulated oocyst achieved with the 3 preparations ranges between 51.4% - 77.5% (Table 4.2). Although highest number of unsporulated oocyst was obtained with 10g of all powder extracts tested, an efficacy range of 47.5% - 66.5% was obtained with the 3 preparations when oocyst were exposed to either extracts at 5g concentration. However, the efficacy of *A. sativum* and *C. papaya* when used in synergy was generally higher than *C. papaya* when used singly, *A. sativum* alone achieved higher efficacy than when in synergy with C. papaya at 2.5g, 5g and 10g concentration. Amprolium, a synthetic coccidiostat achieved highest efficacy than all the preparations at 1hr, 18hr and 48hrs of exposure with 74%, 84% and 91% unsporulated oocyst respectively when given at recommended dose of 1.5g in drinking water. Statistical analysis shows that, though there are variations in the efficacy of the preparations, the difference was not statistically significant at a given concentration (P> 0.05); however there was a significant difference when compared with Amprolium (P≤ 0.05).

Table 1: Effect of aqueous *Allium sativum* and *Carica papaya* on Eimeria oocyst viability

		Exposure Time		
		After 1Hour	**After 24Hours**	**After 48Hours**
Treatment	Conc. g/L	% of oocyst viability	% of oocystviability	% of oocystviability
A. sativum	2.5g	51%	57%	59%
	5.0g	54%	59%	68 %
	10g	57%	64%	79%
C. papaya	2.5 g	46%	48%	50%
	5.0 g	49%	52%	59%
	10.0 g	54%	60%	68%
C. papaya + *A. sativum*	2.5g	49%	53%	55%
	5.0g	53%	56%	66%
	10.0	57%	63%	76 %
Amprolium	**1.5 g**	**74%**	**84%**	**91%**

Table 2: Effects of *Allium sativum* and *C. papaya* powders on oocyst viability

		Exposure Time		
		After 1Hour	**After 24Hours**	**After 48Hours**
Treatment	Conc. g/L	% of oocyst viability	% of oocystviability	% of oocystviability
A. sativum	2.5g	49.1%	53%	56%
	5.0g	53.4%	57.5%	66.5%
	10g	56%	63%	77.5%
C. papaya	2.5 g	44%	46.5%	49%
	5.0 g	47.5%	49.3%	55%
	10.0 g	51.4%	57%	65%
C. papaya + *A. sativum*	2.5g	48.9%	50%	53.7%
	5.0g	52%	53%	64%
	10.0	55%	60%	71%
Amprolium	**1.5 g**	**74%**	**84%**	**91%**

DISCUSSION

In poultry industry, coccidiosis constitutes a major health problem and has been primarily controlled by the use of standard medication under field conditions in spite of limitations like drug resistance and other concerns in relation to food chain contamination. As a substitute, plants and their products have traditionally been used as immune modulators and therapeutics (Nghonjuyi *et al*., 2015a). The in vitro study revealed that both *Allium sativum* and *Carica papaya* extracts induced anticoccidial effect which was concentration dependent and increased by increasing the concentration of the tested extracts (El-Khtam *et al*., 2014and Nghonjuyi *et al*., 2015b).

The increase in efficacy of the aqueous extracts of *A. sativum* and *C. papaya* with increase in concentration efficacy of the extracts could be associated to the variations in concentration of their various phytochemical compounds which probably may require an optimal concentration to enhance the inhibition of the oocyst viability. Lindsy and Blagburn (1995) reported that parasite was highly suppressed when challenged with optimal concentration of active biochemical compound such as phenol and sulphur found in *Aloe vera plant.* Among the two plants evaluated in this study in-vitro, *Allium sativum* was found to exhibit higher efficacy and had the highest percentage reduction of oocysts viability than *Carica papaya*. This confirmed the

findings of El-Khtam *et al*. (2014) who reported that garlic power had high in-vitro anticoccdial effect. This probably may be due to the presence of organosulphur compounds in garlic which are not present in pawpaw. Organosulphur compound has been reported to be highly effective in inhibition of Eimeria parasite (Herrick and Holmes, 1936).Saif *et al*. (2003) recorded high efficacy of sulphur compounds when used as ananticoccidial drug in broiler production. Although the extacts of *A. sativum* and *C. papaya* exhibited anticoccidial property in vitro, Amprolium achieved higher efficacy against *E. tenella* oocyst in vitro. This however is to be expected considering the presence of the chemical compound thiamine analog in Amprolium. Thiamine analog has competitive ability to inhibit the active transport of thiamine in the metabolic cycle. Thus, the coccidian parasites are reported to be several times more sensitive to this inhibition (Richard, 1995).

CONCLUSION

The extracts of the plants *A. sativum* and *C. papaya* may be alternatively use either singly or combine for the control of coccidiosis where orthodox drugs such as Amprolium are not available. The results of this study suggests that supplementation with crude extracts of garlic and pawpaw at concentrations of 10 ml/litre drinking water exhibits a significant anticoccidial activity though not as comparable to that exhibited by Amprolium.

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