In vitro Anthelmintic Activity of *Carica papaya* Leaf and Seed Extracts on Ascarid Eggs

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ABSTRACT

This study was conducted to comparatively assess the In vitro anthelmintic activity of Carica papaya leaf and seed extracts on Ascarid (Ascaridia galli and Ascaris suum) eggs. Five concentrations of methanol and aqueous extracts of the plant parts (3.125 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml and 50mg/ml) were prepared and tested against embryo inhibition of the eggs of Ascaridia galli and Ascaris suum using albendazole as the positive control and 0.1% sulphoric acid solution as the negative control. Phytochemical investigation showed the presence of alkaloids, saponins, fixed oils and reducing sugars of glycosides present in all the crude extracts of Carica papaya. All the crude extracts prepared were able to inhibit embryo in eggs of Ascaris of pigs and poultry. The Interaction of Pawpaw leaf and seed extract at varying concentration on cumulative embryonation of eggs of Ascaridia galli and Ascaris suum in-vitro was highly significant (3.44± 0.183; 3.95± 0.154) values with aqueous seed extracts when compared with albendazole (4.24± 0.000; 3.95 ± 0.000) P<0.05 while the Interaction of Pawpaw leaf and seed extract at varying concentration on cumulative unembryonated egg of Ascaridia galli and Ascaris suum in-vitro also showed a high significant difference (19.69± 0.052;19.54± 0.053) P<0.05 when compared with albendazole (19.43± 0.000; 19.54± 0.000). Furthermore, there was no statistiscal difference (P>0.05) in the interaction of Pawpaw leaf and seed extract at varying concentration on unembryonated egg of Ascaris suumand Ascaridia galli in vitro as all activities on inhibition embryonation were same with increase in concentration from 3.125 mg/ml to 50 mg/ml. The outcome of the current study has provided a scientific justification for the preference of the seeds of Carica papaya for the treatment of helminth infections and has shown that the fixed oils present in the seeds could be responsible for such activity.

Keywords: *Carica papaya, seed, leaf, Ascaridia galli Ascaris suum embryonated, unembryonated, Phytochemical, Pigs, Poultry, Extracts, Concentrations, Scientific, Treatment, Albendazole, Inhibition, In vitro*

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INTRODUCTION

Domestication is an endless process by which animals become adapted to both humans and captive conditions. Cattle, sheep, goat, pigs, poultry and dogs are among the most important domesticated animals in the world (Fabrice, 2019). Cattle, sheep and goat, provide additional sources of meat, milk, fat, farm energy and dung (Wilson, 1991; Thornton, 2010), while poultry, provide nutritional eggs and meat (Junaidu *et al.,* 2014; Gilbert *et al*., 2015). Despite the aforementioned, it has been established that gastrointestinal parasites including major helminthes such

as *Ascaris spp, Ascaridia spp, Raillientina spp, Echinobothridla spp, Heterakis spp, Ancylosloma spp, Uncinaria spp, Stenocephala spp, Toxocara spp, Trichuris spp., Dipylidium spp, Monieza spp, Nematodirus spp,* and protozoans such as *Cryptosporidium spp* and *Entamoeba spp.*, are common pathogens inhabiting the digestive tracts of domesticated animals (Ekpo *et al.,*2010; Adegbulu *et al*., 2015). Infection with these parasites serve as a major. Constraint to animal well-being and productive performance (Regassa *et al.,* 2006; Adedipe *et al.,* 2014). Lowered fertility, reduced work capacity, involuntary culling, reduction in food intake and lower weight gains, reduction in lower milk production, treatment costs, and mortality in heavily parasitized animals are some of the ways gastrointestinal parasites inflict animal health and production (Regassa *et al.,* 2006). Predisposing factors of gastrointestinal parasite infections are grazing habits, climatic condition (temperature, rainfall and humidity), nutritional deficiency, and poor management practice (improper care of animals, unhygienic environment e.t.c), pasture management and immunological status of the host. Losses due to gastrointestinal tract (GIT) parasitism can be classified as direct or indirect in effect (Zingare et al, 2018). Direct losses are due to acute illness and death, forced premature slaughter and rejection of parts of the carcass at meat inspection in abattoirs. Acute parasitic conditions can be recognized, and affected animals are generally treated by the farmer and thus direct losses can be avoided (Zingare et al, 2018). In contrast, sub-clinical levels of parasitism cause indirect and more subtle losses and do not induce obvious clinical signs (Barger *et al.,* 1994). Mortality is low in healthy hosts, but infection is often life-threatening to individuals with poor immunity. Hence, helminth parasites are of significant concern to public health and food safety (Ameen et al, 2010). The frequent use of anthelmintics over many years has inevitably led to the development of drug resistance to one or more of the widely used anthelmintics (Ameen et al, 2018). The extensive use of drugs like albendazole, avermectin, levamisoles, etc, in sub-optimal doses, frequent treatment, under dosing, using lesser drug quality, frequent usage of the same group of anthelmintic for prophylactic, mass treatment of domestic animals and frequent and continuous use of a single drug, have contributed to the widespread development of anthelmintic resistance in helminthes (Ameen et al, 2018). Anthelmintic resistance is a heritable change in a population of worm that enables them to survive drug treatments that are generally effective against the same species at the same dose rate (Terzungwe et al., 2018). Anthelmintic drug resistance has led to a serious problem hindering the successful control and prevention of gastrointestinal nematodes in ruminants and pigs all over the world especially in developing countries, and this has led to a search for alternatives in controlling helminth infection (Piontak et al., 2022). For centuries now, indigenous medicinal plants have been used for the control of internal parasites although, there is no scientific validation of these traditional practices (Effendy et al, 2014., Tchoffo et al, 2019). However, nowadays, Phyto-therapeutic studies on the effects of various plant extracts on pathogenic microorganisms are intensively developing because of the huge plant biodiversity (Piontak et al, 2022). Several natural plant compounds have been extracted by decoction or other simple procedures and they have some advantages like, low cost, easy integration into local communities mostly when plants are locally available and mitigation of the problem of drug resistance (Singh *et al*., 2021).

Carica papaya Linn (Family: Caricaceae), is an herbaceous, perennial, single- stemmed fruit tree plant also called pawpaw, readily available all year round, with easy access for exploitation in tropical areas (Bi and Goyal, 2012., Fasae and Afolabi, 2016). Different parts of those plants have been used for the treatment of various ailments such as those caused by bacterial, helminth and coccidia (Fasae and Afolabi, 2016). The plant contains many biologically active compounds like papain and chymopapain (Agyare et al, 2014). The concentration of the compounds varies in the fruit, latex, leaves and roots. It has been reported to possess high content of protein and good source of minerals with the presence of high fat (Bi and Goyal, 2012), which if adequately harnessed can be beneficial to livestock. The aim of this research was to comparatively assess the *In vitro* anthelmintic activity *of Carica papaya* leaf and seed extracts on Ascarid eggs of pigs and poultry.

MATERIALS AND METHODS

The study was carried out at the Helminthology Laboratory of the Department of Parasitology and Entomology, Faculty of Veterinary Medicine Ahmadu Bello University, Zaria.

Preparation of plant materials

Plant materials and their preparation: *Carica papaya* leaf and *Carica papaya seed* will be collected from fruit sellers and pawpaw farmers within the Ahmadu Bello University Campus areas. They will be air-dried at ambient temperature for one week before they will be broken into tiny pieces with a grinding mill.

Collection and preparation of the *C. papaya* seeds

The seeds were collected freshly from ripe pawpaw fruits from surrounding areas within the campus and washed with clean water to remove dirt and other contaminants. The seeds were air dried at room temperature and grinded into a powdery form. The pawpaw seed powder of 75 g will be blended into liquefaction in 150 mL of distilled water. The mixture was centrifuged at 1,500 rpm. The supernatant was filtered through sterile filter paper

into a conical flask as the study extract. A 1 ml of filtrate is expected to contain 0.5 g (500 mg/ml) of the active ingredients of the *Carica* seed powder (Ameen *et al*., 2018).

Preparation of extract of leaves

10 gm of powder was mixed with 100 ml of distilled water and then the mixture was stirred with a magnetic stirrer at 600 rpm for an hour and then left overnight. The mixture was then filtered, and condensed into 10 ml by the evaporation of solvent in a water bath at 50-60°C. This condensed extract was preserved as a stock solution in refrigerator at 4°C until their use. The methanol extract was prepared following the same procedure by mixing with methanol instead of distilled water (Ameen *et al,* 2018).

Yield Percentage of Extracts

After drying, the yield of each extraction was measured separately and the extraction efficiently was quantified by determining the weight of each of the extracts and the yield percentage was then calculated as dry weight/dry material x 100 (Parekh and Chanda, 2007a)

Phytochemical Determination

The plant fractions were screened for their phytochemical constituents to determine the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates, steroids, anthraquinones, cardiac glycosides and terpenoids using standard phytochemical screening procedures.

Test for Alkaloids

6 ml of extract was mixed with 6 ml of 1% HC1 in steam bath, and then it was filtered. 1 ml of Mayer's reagent was added. Presence of turbidity shows presence of alkaloids. Further addition of a few drops of olive oil to form an emulsion confirmed the presence of alkaloids.

Test for Saponins

0.5 g of the extract was dissolved in 5 ml distilled water. The mixture was shaken vigorously. Formation of stable persistent froth shows the presence of saponins. A further addition of 6 drops of olive oil while shaking forms an emulsion, confirming the presence of saponins.

Test for Tannins

0.5 g of the extract was dissolved in 10 ml of distilled water, then a few drops of 1% ferric chloride solution was added to obtain a brownish green or blue- black precipitate, which confirms the presence of tannin.

Test for Anthraquinones

Borntrager's test was used for the detection of anthraquinones, 0.5g of each extract was taken into a dry test tube and 5ml of chloroform was added and shaken for 5 minutes. The extract was filtered, and the filtrate shaken with an equal volume of 100% ammonia solution. A pink violet or red colour in the ammoniacal layer (lower layer) indicated the presence of free anthraquinones.

Test for Cardiac Glycoside

100mg of the extract was dissolved in 70% alcohol and filtered. About 3 drops of lead sub-acetate was introduced into the filtrate and filtered. The filtrate was extracted with lOmls of chloroform in a separating funnel and concentrated to dryness. The resulting residue was dissolved in 1 ml of glacial acetic acid containing one drop of Ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring obtained at the interphase indicates the presence of a de-oxy-sugar characteristic of cardenolides.

Test for steriods

About 100 mg of the extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interphase was indicative of the presence of steroidal ring.

Test for Terpenes

A little quantity of each extract was dissolved in chloroform, and 1ml of acetic anhydride was added, then two drops of concentrated Sulphuric acid was added. A pink colour which changes to bluish green on standing was indicative of the presence of steroid and terpenes.

Test for Flavonoids

5 ml dilute ammonia was added to 5 ml extract and then 5 ml concentrated sulfuric acid was added. Formation of yellow colour shows the presence of flavonoids.

Test for Carbohydrates

1 gm of the extract was dissolved in 10 ml of distilled water. This extract was boiled with Fehling solution A and B in test tube and colour changes were observed. Presence of brick red colour indicated the presence of reducing sugar.

Test for Phenols

2 ml of extract was dissolved in 4 ml of distilled water and added few drops of 10% FeC13. Appearance of blue or green colour indicates presence of phenols.

Preparation of different concentration of extract

Solutions of these concentrations were prepared using Phosphate Buffer Saline (PBS) as a base (Ameen *et al,* 2018).

Collection of eggs of *Ascarid*

Intestinal samples were collected from the pig and poultry at the various slaughter houses in Zaria and brought to the Helminthology Laboratory of the Ahmadu Bello University Teaching Hospital where the adult worms were collected following a standard method (Fowler, 1990). The adult female *Ascarid*worms collected was crushed gently in mortal, washed with 0.5M KOH solution and filtered into a beaker. They were agitated gently in the 0.5M KOH solution for 30 minutes in order to dissolve the sticky albuminous layer and allow for uniform sampling. The preparation was placed in centrifuge tube and centrifuged at 1500 rpm for 3 minutes to recover the eggs. The supernatant was decanted and the eggs washed three times with distilled water and also with embryonating fluid (0.1M sulphuric acid) for the same period. Volume of the sediment was adjusted to 20 ml in a graduated tube. Using a hypodermic needle, 0.2 ml of the sediment was placed on a McMaster slide (Webster Scientific International, England) for egg count under light microscope. The number of eggs in the remaining volume of the sediment was computed using the number of eggs in 0.2 ml.

***In vitro* screening of *Carica papaya* leaf and seed methanol and aqueous extracts for anthelmintic properties after embryonation (Evaluation of egg inhibition efficacy of the extracts after 24 and 48 hours)**

The *In vitro* evaluation of *Carica papaya* leaf and seed extract on the inhibition of *Ascaridia galli* and *Ascaris suum* embryonated eggs was conducted according to the method described by Gill *et al.* (1995) and Coles *et al.* (2006). Briefly, about 100 *A. galli* and *A.suum* embryonated eggs in 200 µl of water were pipetted into each of 96 wells of microtitre plate respectively. The *in vitro* inhibitory activities of the methanol and aqueous extract of leaf and seed of *C. papaya* were evaluated at five different concentrations of 3.125, 6.25, 12.5, 25, and 50 mg/ml for the two different species respectively. Also, Albendazole was used as standard (positive) control. All tests were performed in triplicates. The plate was covered with foil paper and observed for twenty four (24) and forty eight (48) hours. Similarly, 0.1 H_2SO_4 was employed as negative control while albendazole 50mg/ml served as the positive control standard drug. The plates were kept in the incubator and observed. Thereafter, aliquots of 100 µl from each well were pipetted onto a clean glass slide, for examination at×10 under a light microscope. The numbers of embryonated and unembryonated eggs were counted. The percent inhibition (PI) was estimated using the following formula (Coles et al., 1992): The percentage inhibition of eggs enbryonation was calculated using the formula

$$\text{P\_unembryonated} = \text{eggs} \times \frac{100}{\text{P total}} \quad 1$$

Where P unembryonated eggs= Mean of number of unembryonated eggs in each well
P total = Mean number of eggs in each wells.

***In vitro* egg embryonation inhibition assay**

The *In vitro* evaluation of *Carica papaya* leaf and seed extract on the inhibition of *Ascaridia galli* and *Ascaris suum* egg embryonation was conducted according to the method described by Gill *et al.* (1995) and Coles *et al.* (2006). Briefly, about 200 *A. galli* and *A.suum* eggs in 200 µl of water were pipetted into each of 96 wells of microtitre plate respectively. The *in vitro* inhibitory activities of the methanol and aqueous extract of leaf and seed of *C. papaya* were evaluated at five different concentrations of 3.125, 6.25, 12.5, 25, and 50 mg/ml for the two different species respectively. Also, Albendazole was used as standard (positive) control. All tests were performed in triplicates. The plate was covered with foil paper and incubated at 37ºC for 21 days. The plate was observed on a weekly basis and extracts were added when needed to avoid desiccation. Similarly, 0.1 H_2SO_4 was employed as negative control. The plates were then incubated at 37ºC for 21 days (Coles *et al.*, 1992). Thereafter, aliquots of 100 µl from each well were pipetted onto a clean glass slide, for examination at×10 under a light microscope. The numbers of embryonated and unembryonated eggs were counted. The percent inhibition (PI) was estimated using the following formula (Coles et al., 1992): The percentage inhibition of eggs enbryonation was calculated using the formula:

$$\text{P\_unembryonated} = \text{eggs} \times \frac{100}{\text{P total}} \quad 1$$

Where P unembryonated eggs= Mean of number of unembryonated eggs in each well
P total = Mean number of eggs in each wells.

Statistical Analysis

Data were collected on embyonation and unembryonation rate at 24h and 48h respectively. Embryonation and Unembryonation at these different time intervals were calculated and transformed using square root transformation.

Table 1. Phytochemical constituents and percentage yield detected in the crude methanolic and aqueous extracts of leaf and seed of *Carica papaya*.

Constituents	Pawpaw leaf Aqeuous	Pawpaw leaf Methanolic	Pawpaw seed Aqeuous	Pawpaw seed Methanolic
Alkaloids	+++	+++	+++	++
Saponins	++	++	-	-
Tannins	++	+++	++	-
Flavonoids	++	++	++	-
Carbohydrates	++	-	-	-
phenols	+++	+++	-	-
Steroids	+++	-	+++	++
Anthraquinones	++	-	+++	-
Cardial glycosides	-	+	+++	++
Terpenoids	+	+++	-	-
Percentage yield	33.56	19.08	14.06	3.98

Where - = absent, + = slightly present ++ = more present +++ =highly present
Percentage yield of pawpaw leaf aqeuous extract (%) = 16.78x 100 = 33.56 % 50
Percentage yield of pawpaw leaf methanolic extract (%) = 9.54x100 = 19.08 % 50
Percentage yield of pawpaw seed aqeuous extract (%) = 7.03x100 = 14.06 % 50
Percentage yield of pawpaw seed methanolic extract (%) = 1.99x100 = 3.98 % 50

Table 2: Interaction of Pawpaw leaf and seed extract at varying concentration on cumulative embryonation egg after 48 hours in*Ascaridia galli in vitro.*

	Extracts			
Conc. (mg/ml)	**Aqueous leaf**	**Aqueous seed**	**Methanol leaf**	**Methanol seed**
3.125	19.64a ± 0.052	18.73b ± 0.006	17.90c ± 0.032	16.88d ± 0.109
6.25	16.28e ± 0.533	14.23g ± 0.019	16.41e ± 0.014	15.01f ± 0.145
12.5	12.75h ± 0.166	11.39j ± 0.002	13.97g ± 0.121	11.90i ± 0.055
25.0	7.70l ± 0.213	6.05n ± 0.094	9.03k ± 0.169	6.32m ± 0.089
50.0	4.24o ± 0.000	3.44p ± 0.183	4.29o ± 0.212	4.24o ± 0.142
Albendazole	4.24o + 0.000	2.51.c ± 0.000	3.94p ± 0.000	3.67a ± 0.000
Control	20.05a ± 0.000	17.09d ± 0.000	17.09c ± 0.000	17.09a± 0.000

Means followed with same letter(s) within same column and row are not different statistically at *P>0.05* level of probability using SNK

All data collected were subjected to analysis of variance (ANOVA) using statistical analysis software (SAS Version 9.0). Differences among treatment means were separated using Student Newmann Keuls (SNK) at 5% level of probability.

RESULTS

Aqueous leaf extracts contain alkaloids saponins tannins steroids anthraquinones carbonhydrates and no cardiac glycosides. It also has the highest percent of phytochemical constituents (33.56%) (Table 1). The methanol leaf extract contains all but two (steroids and anthraquinones) of those found in aqueous leaf extract. Saponins, phenol, carbonhydrates and terpenoids are absent in aqueous seed extracts while methanol seed extracts contain only three constituent (alkaloids, steroids and cardiac glycosides) and has the least percentage of constituents (3.98%) (Table 1).

The result of Interaction of Pawpaw leaf and seed extract at varying concentration on cumulative embryonation egg inhibition of *Ascaridia galli in vitro* are presented in (Table 2). In the cumulative period, aqueous seed extract (3.44 ± 0.183) recorded significantly ($P \leq 0.05$) higher inhibition of embryo of *A. galli* than albendazole (4.24 ± 0.000) and other extracts. The number of cumulated embryo recorded in aqueous leaf 4.24 ± 0.000, methanol (4.29 ± 0.212) leaf extracts and methanol seed (4.24 ± 0.142) were statistically ($P \geq 0.05$) similar, but significantly ($P \leq 0.05$) different from aqueous seed extracts (3.44 ± 0.183). At concentration of 3.125 mg/ml, the inhibition of embryonation caused by aqueous leaf was significantly ($P \leq 0.05$) lower than what was obtain from the other treatment groups (19.64 ± 0.052) while at the concentration of 50mg/ml, the inhibition of embyonation caused by aqueous seed recorded the highest.

The mean interaction of pawpaw leaf and seed extract at varying concentration on cumulative unembryonated egg of *Ascaridiagalli in vitro* are presented in (Table 3). The least inhibition of embryonation was recorded for eggs in the control treatment group (1.41 ± 0.000). Aqueous seed extracts (19.69 ± 0.052) produced the highest inhibition of embryonation followed by albendazole (19.43a ± 0.000) and other treatments which were statistically similar (19.43 ±0.000, 19.43± 0.053, 19.41 ± 0.74) to albendazole. The extracts and albendazole produced inhibition of embryonation in a concentration dependent fashion.

The mean interaction of pawpaw leaf and seed extract at varying concentration on cumulative unembryonated egg of *Ascaridia galli in vitro* are presented in (Table 4).

Table 3: Interaction of Pawpaw leaf and seed extract at varying concentration on cumulative unembryonated egg after 48 hours in *Ascaridiagalli in vitro*.

	Extracts			
Conc. (mg/ml)	Aqueous leaf	Aqueous seed	Methanol leaf	Methanol seed
3.125	3.62o ± 0.172	6.60n ± 0.165	6.94m ± 0.046	8.04l ± 0.104
6.25	10.18j ± 0.683	13.04h ± 0.110	9.34k ± 0.384	11.25i ± 0.242
12.5	15.01f ± 0.137	15.84e ± 0.052	13.73g ± 0.110	15.58e ± 0.023
25.0	18.12c ± 0.087	18.54b ± 0.059	17.54d ± 0.090	18.36bc ± 0.060
50.0	19.43a ± 0.000	19.69a ± 0.052	19.41a ± 0.74	19.43a ± 0.053
Albendazole	19.43a ± 0.000	20.51p ± 0.040.	20.32b ± 0.81	20.69a ± 0.009
Control	1.41o ± 0.000	1.41a ± 0.000	1.41w ± 0.000	1.41p ± 0.000

Means followed with same letter(s) within same column and row are not different statistically at *P>0.05* level of probability using SNK

Table 4: Interaction of Pawpaw leaf and seed extract at varying concentration on inhibition of embryonation egg of *Ascaridiagalli in vitro* after 48 hours.

	Extracts			
Conc. (mg/ml)	Aqueous leaf	Aqueous seed	Methanol leaf	Methanol seed
3.125	1.99d + 0.215	1.58e + 0.000	5.05a + 0.099	3.94b + 0.127
6.25	1.58e + 0.000	1.41f + 0.178	3.81b + 0.131	2.55c + 0.000
12.5	0.71h + 0.000	0.71h + 0.000	1.99d + 0.125	1.41f + 0.178
25.0	0.71h + 0.000	0.71h + 0.000	1.22g + 0.000	0.71h + 0.000
50.0	0.71h + 0.000	0.71h + 0.000	0.71h + 0.000	0.71h + 0.000

Means followed with same letter(s) within same column and row are not different statistically at *P>0.05* level of probability using SNK

Table 5: Interaction of Pawpaw leaf and seed extract at varying concentration on unembryonated egg after 21 days in*Ascaridia galli in vitro.*

	Extracts			
Conc. (mg/ml)	**Aqueous leaf**	**Aqueous seed**	**Methanol leaf**	**Methanol seed**
3.125	9.84d + 0.029	9.92c + 0.000	8.69h + 0.058	9.25g + 0.054
6.25	9.92c + 0.000	9.94bc + 0.029	9.30f + 0.054	9.72e + 0.000
12.5	10.02a + 0.000	10.02a + 0.000	9.84d + 0.029	9.94bc + 0.029
25.0	10.02a + 0.000	10.02a + 0.000	9.97b + 0.000	10.02a + 0.000
50.0	10.02a + 0.000	10.02a + 0.000	10.02a + 0.000	10.02a + 0.000

Means followed with same letter(s) within same column and row are not different statistically at *P>0.05* level of probability using SNK

Table 6: Interaction of Pawpaw leaf and seed extract at varying concentration on cumulative embryonationof egg after 48 hours in *Ascaris suum in vitro.*

	Extracts			
Conc. (mg/ml)	Aqueous leaf	Aqueous seed	Methanol leaf	Methanol seed
3.125	19.27c ± 0.054	19.72b ± 0.127	18.60d ± 0.058	17.34e ± 0.068
6.25	15.75g ± 0.034	16.28f ± 0.153	17.41e ± 0.068	15.49h ± 0.185
12.5	12.51k ± 0.051	13.35j ± 0.109	15.19i ± 0.044	12.15l ± 0.245
25.0	7.93o ± 0.225	8.30n ± 0.225	9.98m ± 0.223	6.99p ± 0.080
50.0	4.64q ± 0.127	3.95r ± 0.154	4.77r ± 0.123	4.10q ± 0.148
Albendazole	3.95r ± 0.000	2.87p ± 0.009	3.11q ± 0.871	5.06e ± 0.304
Control	20.05a ± 0.000	20.05a ± 0.000	20.05x ± 0.000	20.05m ± 0.000

Means followed with same letter(s) within same column and row are not different statistically at *P>0.05* level of probability using SNK

There were no significant differences in all the groups at a concentration of 50mg/ml. The least inhibition of embryonation was recorded in all groups at a concentration of 3.125 mg/ml. Interaction of Pawpaw leaf and seed extract at varying concentration on unembryonated egg of *Ascaridia galli in vitro* are presented in (Table 5). The interaction of aqueous leaf extract at 12.5mg/ml was similar and higher (10.02 + 0.000) as compared with 6.25 and 3.125mg/ml, however, 6.25mg/ml (9.92c + 0.000) was higher than 3.125mg/ml (9.84d + 0.029). However, from 25mg/ml, all the treatment groups had no significant difference (P>0.05). Interaction

Table 7: Interaction of Pawpaw leaf and seed extract at varying concentration on cumulative unembryonated egg after 48 hours in*Ascaris suum in vitro.*

	Extracts			
Conc. (mg/ml)	**Aqueous leaf**	**Aqueous seed**	**Methanol leaf**	**Methanol seed**
3.125	4.64k + 0.127	3.79l + 0.653	5.95j + 0.095	7.60i + 0.073
6.25	10.01h + 0.209	10.58g + 0.308	7.53i + 0.073	9.93h + 0.413
12.5	14.99d + 0.029	14.61e + 0.111	12.44f + 0.135	15.26d + 0.218
25.0	17.80b + 0.89	18.06b + 0.116	17.04c + 0.119	17.87b + 0.064
50.0	19.27a + 0.054	19.54a + 0.053	19.22a + 0.054	19.49a + 0.053
Albendazole	19.54a + 0.000	19.27p ± 0.111	19. 15p ± 0.423.	18.97p ± 0.123
Control	1.41m + 0.000	1.41d ± 0.000	1.41z ± 0.000	1.41i ± 0.00

Means followed with same letter(s) within same column and row are not different statistically at *P>0.05* level of probability using SNK

Table 8: Interaction of Pawpaw leaf and seed extract at varying concentration on embryonation egg after 48 hours in*Ascaris suum in vitro.*

	Extracts			
Conc. (mg/ml)	**Aqueous leaf**	**Aqueous seed**	**Methanol leaf**	**Methanol seed**
3.125	2.65g + 0.095	2.45h + 0.102	9.54a + 0.079	7.78c + 0.064
6.25	2.12i + 0.000	1.73j + 0.145	7.94b + 0.094	4.58e + 0.055
12.5	1.73j + 0.145	0.71k + 0.000	4.74d + 0.105	2.91f + 0.172
25.0	0.71k + 0.000	0.71k + 0.000	2.64g + 0.095	2.23i + 0.112
50.0	0.71k + 0.000	0.71k + 0.000	1.73j + 0.145	1.58j + 0.000

Means followed with same letter(s) within same column and row are not different statistically at *P>0.05* level of probability using SNK

Table 9: Interaction of Pawpaw leaf and seed extract at varying concentration on unembryonated egg after 21 days in *Ascaris suum in vitro.*

	Extracts			
Conc. (mg/ml)	**Aqueous leaf**	**Aqueous seed**	**Methanol leaf**	**Methanol seed**
.125	9.69ef + 0.030	9.74def + 0.030	3.13j + 0.241	6.36h + 0.079
6.25	9.82bcd+ 0.000	9.89bc + 0.029	6.15i + 0.124	8.93g + 0.032
12.5	9.89bc + 0.029	10.02a + 0.000	8.86g + 0.056	9.62f + 0.052
25.0	10.02a + 0.000	10.02a + 0.000	9.67ef + 0.030	9.79cde + 0.029
50.0	10.02a + 0.000	10.02a + 0.000	9.89bc + 0.029	9.92ab + 0.000

Means followed with same letter(s) within same column and row are not different statistically at *P>0.05* level of probability using SNK

of Pawpaw leaf and seed extract at varying concentration on cumulative embryonation egg of *Ascaris suum in vitro* (Table 6). The interaction of Pawpaw leaf and seed extract at different concentration on cumulative embryonation inhibition of *Ascaris suum* eggs shows that there were significant (P≤0.05) decrease in embryo in eggs of *A. suum* with increase in concentration of the various extract. Application of 3.125mg/ml of aqueous (19.72 ± 0.127) extract resulted in higher number of embryonated eggs that were significantly (P≤0.05) different from other treatment, while 50mg/ml of aqueous seed (3.95 ± 0.000) extract had significantly (P≤0.05) lower number of embryonated eggs than other treatment combinations. However, application of 50mg/ml of aqueous seed extract resulted in higher number of inhibited embryo that was statistically (P≥0.05) comparable to all other extracts at the same concentration, but significantly (P≤0.05) different from other treatment combinations.The result of interaction of pawpaw leaf and seed extract at varying concentration on cumulative inhibition embryonation egg of *Ascarissuum in vitro* are presented in (Table 7). In the cumulative period, aqueous seed extract (19.54 + 0.053) and albendazole (19.54 + 0.053) recorded significantly (P≤0.05) higher inhibition of embryo of *A.suum* than other treatment group (19.27 + 0.054, 19.22 + 0.054, 19.22 + 0.054), that were at par. The number of cumulated embryo recorded at 25mg/ml in aqueous leaf 17.80 + 0.89 methanol (17.04 + 0.119) leaf extracts and methanol seed (17.87 + 0.064) were statistically similar, but significantly (P≤0.05) different from aqueous seed extracts (3.79l + 0.653). At a concentration of 3.125mg/kg, embryonation inhibition caused by aqueous leaf was significantly (P≤0.05) lower than what was obtain from the other treatment groups (4.64 + 0.127, 5.95 + 0.095, 5.95 + 0.095).

The mean interaction of pawpaw leaf and seed extract at varying concentration on cumulative unembryonated egg of *Ascarissuum in vitro* are presented in (Table 8). There were no significant differences in all the groups at a concentration of 50mg/ml. The least inhibition of embryonation was recorded in all groups at a concentration of 3.125 mg/ml. Interaction of Pawpaw leaf and seed extract at varying concentration on

unembryonated egg of *Ascaris suum in vitro* are presented in (Table 9). The interaction of aqueous seed extract at 12.5mg/ml was similar (10.02 + 0.000) as compared with 6.25mg/ml, and higher than 3.125mg/ml. Also, 6.25mg/ml (9.92c + 0.000) was higher than 3.125 (9.84d + 0.029). However, from 25mg/ml, all treatment groups had no significant difference (P>0.05).

DISCUSSION

The phytochemical analysis showed the presence of alkaloids, tannins, flavonoids and saponins in the methanol and aqueous extracts of *Carica papaya* seeds and leaf, also glycosides and reducing sugars were present in the methanol and aqueous extracts of seeds and leaf. This observation is consistent with the findings of Naggayi *et al.,* 2015, who revealed the presence of saponins, glycosides, tannins, flavonoids and alkaloids in the aqueous extract of seeds. Another observation which is not consistent with earlier reports observed that flavonoids, glycosides and saponins were present only in ethanol extract of *Carica papaya* leaves, whereas tannins were present in the n-hexane extract of the leaves which contributes to the bioactivities of the leaf extracts (Dewair and Bessat, 2022). However, Goku *et al., (*2020) observed that tannins were observed only in extracts of the leaves while fixed oils on the other hand were only present in the extracts of the seeds. The *in vitro* anthelminthic investigations, indicated that the effects of the extracts on inhibition of embryonation of ascarid eggs concentration dependent, while the present study observed an increasing effect of the extracts with increasing concentration from 3.125 mg/ml to 50 mg/ml. Both methanol leaf and aqueous seed extracts at 50 mg/kg showed potent value in the treatment of ascariosis in pigs and poultry, and irrespective of solvent used to extract the active ingredients, which encourages further investigation of their use as therapeutic agents against *Ascaris suum* and *Ascaridia galli* infections in endemic regions. This study showed that methanol and aqueous extracts of *Carica papaya* leaf and *Carica papaya* seed have anthelminthic activity against larval and ova stages of *Ascaris suum* and *Ascaridia galli.* The magnificent worth of this plant is demonstrated by the fact that the results obtained with the extracts on helminths are similar to those of commercially available patented anthelminthic drug Albendazole. Ameen *et al, (*2010) made a similar report in west African dwarf goats, were he observed that *Carica papaya* seed was therapeutic against natural helminthic infection in goats when compared with thiabendazole. Other research publications on non-ruminants has suggested that the latex and seeds of the *Carica papaya* have potential anthelmintic effects on helminths in mice, rats, pigs, and poultry (Bi and Goyal, 2012., Singh et al, 2022). According to Dewair and Bessat, (2022), the anthelmintic properties of *C. papaya* seed extracts are related to the presence of benzyl isothiocyanate. Again, anthelminthic property may be attributed to the presence of papain in the seeds and leaf of *C papaya* and this is possible because the papain is capable of digesting bacteria and parasitic cells, hence its use as an anthelmintic and antibiotics as reported by (Tchoffo et al, 2019). In this study the compound found in the extract are similar to those found in the commercial preparation of albendazole (benzimidazole). This can be used as an acaricide. In an *in vitro* study, Kumar *et al.* (1991) compared effects of BITC (benzylisothiocyanate), an anthelmintic principle of *Carica papaya* with mebendazole against *A. galli* and found it to be effective and consistent to the observations of this present work. Dakpogan (2005) stated anthelmintic property might be due to presence of proteolytic enzymes such as papain, chymopapain and lysozymes in the latex as well as in leaves of the plant. The present study has shown that aqueous seed extracts was effective against development of *A. galli, A. suum* larvae and ova. This study also indicated that methanolic leaf extracts had a better efficacy than the aqueous extract. This may be explained that the active ingredients of papaya leaves are more soluble in alcoholic extract than water, which suggest why papaya leaves have strong efficacy against the development of *A. galli* and *A. sum* eggs in methanol extracts (Singh et al, 2022). The seed of *Carica papaya* were similar to thiabendazole in terms of their activity against gastrointestinal helminths in Sokoto Red goats, according to a prior study by (Ameen *et al,* 2018). Dewair and Bessat, (2022) indicated that the seeds, stem bark and leaves were both safe and effective at getting rid of intestinal helminths (Goku *et al.,* 2020). Indicating that *Carica papaya* seeds extract may be used as an alternative to synthetic drugs. The present study also revealed the anthelmintic activity of *Carica papaya* against gastrointestinal helminths and correspond with the report by Ameen *et al. (*2010); Ameen *et al. (*2018); Effendy *el al.,* 2014; Feroza *et al*., 2017; Effendy *el al., (*2014); Feroza *et al*. (2017); Zingare *et al., (*2018). Garcia *et al*, 2019. This may be due to presence of alkaloids, saponins, glycoside and fixed oils. Since some of these phytoconstituents have been documented to possess anthelminthic activities thus may have contributed to the observed activity in this present work. This finding might also support the conventional wisdom that the stem bark, flowers, roots, and seedshave anthelminthic properties in them and have been used in management of several conditions, chiefly among them are their roles in managing helminth infections (Agyare *et al.,* 2014). The findings of this work may be of great advantage to a poor society since the plant is readily available year-round. Phytochemical screening of C. *papaya* leaves and seed extracts revealed abundant secondary chemicals such as tannins, flavonoids, terpenes, and these have been linked to the plant's purported anti-microbial and ethno-medical effects (Terzungwe et al., 2018). The presence of alkaloid, carbohydrate, glycoside, phenols, tannin, saponin, and oxalate shows the greater intensity of their presence in methanolic, ethanolic, hexane, and ethyl acetate extract as reported by Effendy et al. (2014) who demonstrated that

extracts exhibit significant inhibitory activity against *Candida albicans* the causative organism of candidiasis. Another study by Siddiqui *et al,* 2018, revealed that peels of banana and papaya fruits are potentially good source of antioxidant and antibacterial agents. The antimicrobial activity of the plant, which was visible in the antimicrobial activity against the tested organisms used for their study, was caused by these phyto constituents (Piontak et al, 2022). Ethanol extract of *Carica papaya* showed a significant broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria.

Conclusion

The results obtained from this work validated the traditional or herbal practitioners'use *of Carica papaya,* especially the seeds and leaf as an anthelmintic agent in the treatment of helminthosis in pigs and poultry. At a concentration of 50mg/ml *Carica papaya* aqueous seed extract caused inhibition of embryonation of *A.galli and A.suum eggs* which were statistically higher than abendazole and to the other treatment groups. For unembryonated eggs of *A.galli* and *A. suum* aqueous seed extract record was statiscally at par with albendazole and other treatments. Further investigation will be needed to identify the active ingredients responsible for the observed anthelminthic properties of aqueous seed extracts. The anthelmintic efficacy of the seed and leaf extracts of *Carica papaya* should be tested on other helminths of veterinary importance. Further studies be done on other parts of the plant such as the root, stem, peels and latex to evaluate for possible anthelmintic properties

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