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# *In vivo* Anticoccidial and Antioxidant Activities of *Psidium guajava* Methanol Extract

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors YC, VKP, NACN, JRK and MM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NK, EK and ML managed the analyses of the study. Authors EK and ML managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** Coccidioses are the major parasitic diseases in poultry and other domestic animals including the domestic rabbit (*Oryctolagus cuniculus*).The aim of this study was to evaluate *in vivo* anticoccidial and antioxidant activities of *Psidium guajava* methanol extract.
**Materials and Methods:** A total of 48 domestic rabbits (60 days old and about 1.5-2 kg body weight) and free from coccidia infection were used. All groups except group 6 were infected with 1000 sporulated *Eimeria intestinalis* oocysts. Faecal samples were collected and examined starting on day one post-inoculation until Day 9 post-inoculation, during which oocysts appeared in faeces. For quantitative analysis or determination of the number of oocysts per gram (OPG) of faeces, the Mc Master technique was used. Serum was used for determination of biochemical parameters

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related to oxidative stress such as Superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), malondialdehyde (MDA), nitric oxide (NO) and Glutathione using standardized diagnostic kits and a spectrophotometer.
**Results:** The present study revealed that *Psidium guajava* has significant *in vivo* anticoccidial and antioxidant activities and can thus be used to protect tissue from oxidative stress. Oocyst counts in the group treated with 2% DMSO increased continuously from the initial count on Day 0 as opposed to treated groups. The highest oocyst count reduction rate was 95% in the group which received the standard anti-coccidial drug. Among the groups that received the plant extract, the highest oocyst reduction rate was 79.6% at a dose of 500 mg/kg and reduced in a dose-dependent manner to 66.1% (250 mg/kg) and then 56.2% (125 mg/kg). The result therefore showed a significant decrease in tissues and serum catalase and peroxidase, and a significant increase in NO, MDA and GLU levels in negative control animals. Treatment resulted in a significant normalization of the levels of the above markers when compared with the neutral control group.
**Conclusion:** These results therefore provide confirmation to the usage of *Psidium guajava* against coccidioses by Agropastoral farmers in Cameroon.

*Keywords: Psidium guajava; anticoccidial activity; antioxidant activity; Eimeria intestinalis; Coccidiosis*

## 1. INTRODUCTION

The potential for rabbit production in Cameroon is high considering that other sources of meat are often scarce and costly to most families. Consumers prefer rabbits for their low cholesterol and fat contents and high levels of essential amino-acid [1]. In addition to this commercial value, it is also due to the high prolificacy, early maturity, fast growth rate, high genetic selection potential, efficiency in feed conversion and economic utilization of space by rabbits [2]. Therefore, rabbit production became one of the important animal resources in the world [1]. Coccidioses are the major parasitic diseases in poultry and other domestic animals, including rabbits [3]. They negatively affect the financial status of farmers, the environment and food supply. Most of the current anti-coccidial drugs show low efficacy and cause deleterious side effects. Because of widespread drug resistance constraint [4], residual effects of drugs in meat of animals and toxic effects of disinfectants, scientist all over the world are shifting towards alternative approaches for the control of parasitic problems [5]. In various physiological and pathological conditions, the systemic amount of free radicals and reactive oxygen species are higher than normal. Free radical oxidative species are known to be produced during the host's cellular immune response to invasion by *Eimeria* species [6], which plays an important role in defending against parasitic infections.

Georgieva et al. [7] reported that *E. acervulina* oocysts motivate lipid peroxidation, increase oxidative damage and imbalance in the antioxidant status in infected animals by disturbing the oxidative balance. Therefore, to alleviate or reduce the oxidative stress natural (e.g. Vitamin E, Se) and synthetic (e.g. butylated hydroxytoluene) antioxidants as feed supplements are commonly used in the poultry industry.

The use of antioxidants as anticoccidial remedies, therefore, holds promise as an alternative in the control of coccidiosis. Today, the use of antioxidant rich plant extracts has gained special importance because of restriction in the use of synthetic compounds against coccidial infections due to emergence of resistance and their drug residues [8]. Therefore, the use of natural antioxidants may alleviate the difficulties related to synthetic drugs, as they are not only natural products but may comprise new molecules to which resistance has not yet developed. *Psidium guajava* is a medicinal plant used in tropical and subtropical countries to treat many health disorders. It has indeed been variously reported that *Psidium guajava* leaf extract has a wide spectrum of biological activities such as anticough, antibacterial, haemostasis [9], antidiarrhoeal and narcotic properties [10] and antioxidant properties [11]. This work was therefore aimed at evaluating anticoccidial and antioxidant activities of the methanol extract of *P. guajava* in order to justify its usage by Agropastoral farmers as an anticoccidial drug.

## 2. MATERIALS AND METHODS

The study was conducted in the Research Unit of Biology and Applied Ecology for the parasitological parameters and the Research Unit of Microbiology and Antimicrobial

Substances for the biochemical parameters, from February 2016 to March 2016.

## 2.1 Plant Material

Leaves of *Psidium guajava* were collected in Menoua Division, Western Region of Cameroon and identified by Mr. NGANSOP Eric, a botanist at the Cameroon National Herbarium (Yaoundé) using a voucher specimen registered under the Reference No 2884/SRF.

## 2.2 Preparation of Extract

The methanol extract was obtained using the procedure described by Abah and Egwari [12].

## 2.3 Anticoccidial Evaluation of *P. guajava*

The methanol extract of *P. guajava* was used to carry out the *in vivo* test. A total of 48 domestic rabbits (60 days old and about 1.5-2 kg body weight) and free from coccidian *Eimeria intestinalis* oocysts, infection were used. These animals of both sexes i.e. 24 males and 24 females were acclimatized for two weeks and their fecal samples examined daily for 2 successive weeks to confirm that the animals were free from coccidian by the use of the concentration flotation technique before being used for this study. Each rabbit was individually housed in wire-floored batteries under sanitary conditions. Good drinking water and feed were provided *ad libitum* to the animals. The animals were housed in the Experimental Animal House of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang. The experimental groups were arranged as follows:

Group 1: Infected and treated with the extract of *P. guajava* at 500 mg/kg of body weight.
Group 2: Infected and treated with the extract of *P. guajava* at 250 mg/kg of body weight.
Group 3: Infected and treated with the extract of *P. guajava* at 125 mg/kg of body weight.
Group 4: Infected and treated with Amprocox at 5 mg / kg body weight.
Group 5: Infected and treated with 2% DMSO.
Group 6: Non infected – non treated (negative control)

All groups except group 6 were innoculated with 1000 sporulated *Eimeria intestinalis* oocysts.

## 2.4 Parasitological Examination

Faecal samples were collected from each of the above groups and examined starting on day one post-inoculation until day 9 post-inoculation, during which oocysts appeared in faeces. For quantitative analysis or determination of the number of oocysts per gram (OPG) of faeces, the Mc Master technique described by Thienpont et al. [13] was used.

## 2.5 Parameters Studies

### 2.5.1 Oocyst reduction rate

The percentage of oocyst reduction was determined using the formular [14]:

$$Oocyst\ reduction\ rate\ (\%) = \frac{Initial\ mean\ OPG - Final\ mean\ OPG}{Initial\ mean\ OPG} * 100$$

### 2.5.2 Feed consumption

Feed consumption of the animals was determined every day by making the difference between the weight of initial food and that of remaining food.

Wc = Ws– Wr with:

Wc = Weight of feed consumed (g)
Ws = Weight of feed served (g)
Wr = Weight of the remaining feed (g)

### 2.5.3 Average daily weight gain

The average daily weight gained on each day was noted using the formular [14].

Average daily weight gain (AWG) = Total weight gain per rabbit in each group/ Total number of days.

### 2.5.4 Feed conversion ratio

The feed conversion ratio was determined using the formular:

Feed conversion ratio (FCR) = Mean total feed consumed / Total weight gain for a particular period
Where,

- Total feed consumed = cumulative feed from day1 to day 7
- Mean total feed consumed = total feed consumed /no. of animals

- Weight gain = body weight on day 7 – Body Weight on day1
- AWG = Weight gain / No. of animals
- Growth rate (from day1-7) = AWG/ 7 days

#### 2.5.5 Mortality percentage

The percentage of mortality was recorded as follows.

$$\text{.Mortality percentage} = \left(\frac{\text{Number of live rabbits at the end of the experiment}}{\text{Total Numberof rabbits}}\right) * 100$$

### 2.6 *In vivo* Antioxidant Activity of *P. guajava*

This test was performed with rabbits aged 60 days old and about 1.5-2 kg body weight. It was designed to investigate the antioxidant effect of the methanol extract at different doses during an infection with a strain of *E. intestinalis*.

### 2.7 Blood Collection, Serum and Intestine Homogenate Preparations after Treatment

At the end of the treatment, animals were subjected to a 12-hour food fasting and their blood samples were collected by cardiac puncture from chloroform vapors anaesthetized rabbits into sterilized dry tubes. The tubes were allowed to clot for 30-60 min at room temperature and centrifuged at 3000 rpm for 10 minutes at 20°C to obtain serum. The animals were further dissected and their intestines were removed. The harvested tissue (intestine for therapeutic efficacy) was rinsed with phosphate buffered saline (PBS) and blotted with filter paper and weighed. Fifteen percent (15%) homogenate of the intestine was prepared in 0.1 M phosphate buffer, pH 7.4 at the ratio of 15:100 w/v and then centrifuged at 3000 rpm for 15 minutes. The serum was used for the determination of biochemical parameters related to oxidative stress such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), malondialdehyde (MDA), nitric oxide (NO) and glutathione using standardized diagnostic kits (IMNESCO GmbH, Germany) and a spectrophotometer (Schimadzu 1501, Japan).

### 2.8 Evaluation of Biochemical Antioxidant Parameters

#### 2.8.1 Enzymatic parameters

*2.8.1.1 CAT (catalase, EC 1.11.1.6) activity*

Catalase activity was determined in tissue and serum using the method described by Fodouop et al. [15] method with some modifications. A total of 25 µL of the homogenates were added to tubes containing 375 µL of phosphate buffer (pH 7.4) and 100 µL of 50 mmol/L $H_2O_2$. After one minute incubation at room temperature, 1 mL of dichromate (5%) prepared in acetic acid at 1% was added. The mixture was homogenized and incubated at 100°C for 10 min then cooled in ice bath and the absorbance was recorded at 570 nm using Schimadzu 1501 spectrophotometer Japan. One unit of activity is equal to one mmol/L of $H_2O_2$ degraded per minute and is expressed as units per milligram of protein.

*2.8.1.2 POD (Peroxidase EC 1.11.1.7) assay*

Peroxidase activity was determined in tissues and serum using the Habbu et al. [16] method. To 0.5 mL tested sample were added 1 mL of 10 mM potassium iodide solution and 1 mL of 40 mM sodium acetate. The absorbance of potassium iodide was read at 353 nm which indicates the amount of peroxidase. Then 20 µL of $H_2O_2$ (15 mM) was added and the change in the absorbance in 5 min was recorded. Units of Peroxidase activity were expressed as the amount of enzyme required to change the optical density by 1 unit per min. The specific activity was expressed in terms of units per mg of proteins.

#### 2.8.2 Non enzymatic parameters

*2.8.2.1 Malondialdehyde (MDA) assay*

The extent of peroxidation in tissues and serum was assessed by measuring the level of malondialdehyde (MDA) according to the method of Fodouop et al. [15] with some modifications and peroxidation in the tissues was calculated based on the molar extinction coefficient of malondialdehyde (MDA) (153 $mM^{-1}cm^{-1}$), and expressed in terms of micromoles of MDA/g of tissue.

*2.8.2.2 Nitric oxide (NO) assay*

To 340 µL of the experimental sample, 340 µL of freshly prepared 1% sulfanilamide in 5%

orthophosphoric acid were added after 5 min of incubation in the dark at room temperature, 340 µL of the NED (N-(1-Naphthyl) ethylenediamine) Solution (0.1% NED in water) were also added. The resulting solution was well mixed and then incubated at room temperature for 5 mins protected from light. The absorbance of the colored azo compound formed was measured at 520 nm within 30 minutes. A standard curve was plotted using nitrite ($NaNO_2$) (100, 50, 25, 12.5, 6.25, 3.13 and 1.56 µM). The results were expressed as Micromolar of Nitrite Equivalents (µMNE) per gram (g) of tissue or per millilitre (mL) of blood.

*2.8.2.3 Reduced GSH activity*

GSH was determined in tissue by the Oyedemi et al. [17] method with some modifications. A total of 0.8 mL of 0.3 mol/L dihydrate sodium phosphate solution was added to 0.2 mL of homogenate. It was centrifuged at 5 000 ×g for 5 min and 0.5 mL of 0.4 mg/mL dithiobis-nitrobenzoate (prepared in 1% sodium citrate) was added to the supernatant. The optical density was recorded at 412 nm. The total GSH was calculated based on the molar extinction coefficient of GSH (1.36 ×105 mM-1cm-1) and expressed in terms of micromoles of GSH/g of tissue.

## 2.9 Phytochemical Screening

The extracts were tested for presence of phenolic compounds, alkaloids, flavonoids, polyphenols, tannins, saponin, triterpenes and steroids using standard procedures described by Builders et al. [18] in the Laboratory of Microbiology and Antimicrobial Substances.

## 2.10 Statistical Analysis

Results were expressed as mean ± standard error of mean (S.E.M.). Within group, comparisons were performed using ANOVA one way test. Significant difference ($p \leq 0.05$) between control and experimental groups was assessed using Waller Duncan test.

# 3. RESULTS

## 3.1 *In vivo* Anti-coccidial Evaluation of *Psidium guajava*

The final faecal oocyst counts of *Eimeria*-infected rabbits treated with extracts in different groups showed a significant reduction in oocyst count between treatment groups in dose-related manner on day7 (Table 1). Oocyst counts in the 0.2% DMSO treated increased continuously from the initial count on Day 0 as opposed to the plant extract groups. The highest oocyst count reduction rate was 95.9% in the group which received the standard anti-coccidial drug. Among the groups that received the plant extract, the highest oocyst reduction rate was 79.6% at a dose of 500 mg/kg and reduced in a dose-dependent manner to 66.1% (250 mg/kg) and then 56.2% (125 mg/kg).

## 3.2 Effects of the Methanol Extract of *P. guajava* on Growth Parameters

The growth parameters of *Eimeria*-infected rabbits treated with methanolic *P. guajava* extract are presented in Table 2. Results obtained showed that there were significant differences in the feed intake between the different groups receiving the extract ($p < 0.05$). The results of weight gain (Table 2) showed that body weight gain in all the treated groups were significantly ($p<0{\cdot}05$) higher than the infected non-medicated control. Among herbal medicated groups, the maximum weight gain was shown by the group treated with 500 g followed by the groups medicated with 250 and 125 mg/kg of body weight. There was no significant ($p>0{\cdot}05$) difference between weight gain of groups treated with 500 and 250 mg of *P. guajava* extract and amprocox. The FCR values (Table 2) of the treated groups were numerically lower compared with the infected non-treated groups receiving *P. guajava* extract, although a statistical comparison could not be made due to group feeding. Among the medicated groups, the lowest FCR was observed in the group medicated with 500 mg of methanol extract per body weight followed by the groups medicated with 250 and 125 mg/kg.

## 3.3 Effects of the Methanol Extract of *P. guajava* on Mortality Rate

It is clear from the results presented in Table 3 that mortality was higher in the infected non-treated control group compared with treated groups. Among treated groups, mortality was numerically lowest in amprocox treated group compared with the other treated groups. Among the extracts treated groups maximum mortality was observed in 125 mg/kg of *P. guajava* medicated group. While 250 mg /Kg and 500 mg /Kg of *P. guajava* treated groups showed similar mortality.

**Table 1. Reduction rate of *E. intestinalis* oocyst counts per gram of feaces in rabbits treated with methanol extracts of *P. guajava***

| Treatments mg/kg | Oocyst counts at different days of treatment | | | | Oocyst reduction rate % |
|---|---|---|---|---|---|
| | Day0 (Initial) | Day1 | Day4 | Day7(Final) | |
| 500 | 6088.9±1424.13[a] | 5177.76±1376.53[a] | 2022.23±482.26[ab] | 1244.43±328.86[ab] | 79.56 |
| 250 | 6488.9±1387.77[a] | 5800±1400[a] | 3022.23±772.66[ab] | 2200±352.76[ab] | 66.1 |
| 125 | 7311.1±2736.03[a] | 6711.1±2451.6[a] | 4511.1±1645.63[b] | 3200±1156.63[b] | 56.2 |
| ITD | 7577.766±2505.4[a] | 6744.43±2450.46[a] | 10222.23±3166.73[c] | 12377.76±2329.83[c] | -63.34 |
| ITA5 mg/kg | 5977.76±2215.43[a] | 5111.1±1989.23[a] | 1511.1±766.9[ab] | 244.43±234.13[a] | 95.9 |
| NNT | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | |

*ITD: Infected and treated with DMSO, ITA: Infected and treated with Amprocox at 5 mg/kg and NNT: Non infected- non treated. Values are Mean ± SEM. For the same column, values carrying the same superscript letter are not significantly different at p ≥0.05 (Student-Newman-Keuls Test).*

**Table 2. Mean total feed consumed, total weight gain, and feed conversion ratio of rabbits treated with methanol extract of *P. guajava***

| Treatments mg/kg | Growth Parameters | | |
|---|---|---|---|
| | MTFC | TWG | FCR |
| 500 | 358.8 ±5.9[b] | 130.64 ± 4.28[b] | 2.75 |
| 250 | 392 ±2.6[c] | 125.28 ± 1.18[b] | 3.13 |
| 125 | 313.2 ±2.9[a] | 98.28 ± 2.37[a] | 3.22 |
| ITD | 384 ±2.6[bc] | 84.16 ± 2.37[b] | 4.56 |
| ITA 5 mg/kg | 369.2 ±0.6[bc] | 133.68 ± 3.11[b] | 2.76 |
| NNT | 414 ±1.2[d] | 149.28 ± 0.33[b] | 2.77 |

*ITD: Infected and treated with DMSO, ITA: Infected and treated with Amprocox at 5 mg/kg and NNT: Non infected- non treated. Values are Mean ± SEM. For the same column, values carrying the same superscript letter are not significantly different at p ≥ 0.05 (Student-Newman-Keuls Test)*

**Table 3. Effect of treatment onpercentage mortality (n = 8) in Rabbits experimentally inoculated with sporulated oocyts of *Eimeria intestinalis***

| Plant | Groups and doses | Mortality days during treatment | | | | | | | Total mortality | Mortality percentage% |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | |
| *P. guajava* | 125 mg | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 25 |
| | 250 mg | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 12.5 |
| | 500 mg | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 12.5 |
| Positive control | ITA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Negative control | ITD | 0 | 0 | 1 | 1 | 1 | 2 | 0 | 5 | 62.5 |
| Normal control | NNT | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*ITD: Infected and treated with DMSO, ITA: Infected and treated with Amprocox at 5 mg/kg and NNT: Non infected- non treated*

## 3.4 *In vivo* Antioxidant Activities of *P. macrophylla* and *P. guajava* Extracts

### 3.4.1 Enzymatic antioxidant parameters

*3.4.1.1 Effect of treatment on tissues and serum catalase activity*

The evolution of tissue and serum catalase activity as a function of extract doses are shown in Table 4. From this Table, it appears that the infection (ITD) resulted in a significant ($p<0.05$) decrease in the catalase activity compared to normal control. However, treatment with the various doses of plant extract resulted in significantly higher ($p < 0.05$) tissues and serum catalase when compared with the negative control rabbits.

*3.4.1.2 Effect of treatment on total peroxidase (POD)*

The effect of the treatment on POD activity in the tissues and serum are presented in Table 5. The result shows a significantly decreased ($P<0.05$) of POD activities, in the tissues of the negative control animals compared to the normal control groups. However, treatment with only 500mg of plant extract increased the tissues and serum peroxidase activities when compared with the negative control rabbits.

### 3.4.2 Non enzymatic antioxidant parameters

*3.4.2.1 Effect of treatment on malondialdehyde (MDA)*

Generally, the MDA tissues and serum concentrations significantly increased in negative control animals compared to the normal control animals ($p < 0.05$, Table 6). Interestingly animals treated with various doses of plant extract and amprocox had a dramatical decrease in the level of MDA compared to negative control animals. The decrease in MDA levels is a doses-dependent manner in the animals receiving different doses of plant extract.

*3.4.2.2 Effect of treatment on nitric oxide (NO)*

The tissues and serum concentration of NO in control and experimental groups of rabbits as presented in Table 7. As illustrated by Table 7 below, infection resulted in an increase in the tissues and serum NO compared to normal control. Surprisingly, there was a significant reduction of the serum NO level in the different doses of *P. guajava* extract and amprocox treated animals compared with negative control group. A significant decrease in the level of intestinal NO ($p < 0.05$) was noted in 125 and 250 mg of plant extract treated groups compared to negative and normal control.

*3.4.2.3 Effect of treatment on reduced glutathione (GSH)*

The effect of treatment on tissues and serum concentration of GSH are shown in Table 8. The parasitic infection resulted in a significant increase in tissues but not serum GSH compared to normal control group. Even though not significant in the serum, administration of different extracts at different doses caused a significant reduction of tissue GSH compared to the negative control. Furthermore, it seemed that there was a significant dose-dependent decrease in the GSH between 125 and 500 mg plant extract treated groups.

*3.4.2.4 Effect of treatment on superoxide dismutase activity (SOD)*

The effects of plant extract treatment on tissues and serum SOD activities are shown in Table9. Infection resulted in a significant increase in serum but not tissues SOD activity compared to the neutral control. However the plant extract and amprocox treated groups markedly reduced serum SOD activities compared to negative control group.

**Table 4. Effect of treatment on catalase in the serum and tissues**

| Plant | Groups and doses | Serum | Intestine |
|---|---|---|---|
| | | Quantities of catalase (µmol/min/g of tissue or µmol/min/ml of serum) | |
| *P. guajava* | 125 mg | $0.256\pm0.000^{b}$ | $14.317\pm1.289^{b}$ |
| | 250 mg | $0.258\pm0.007^{b}$ | $19.657\pm1.503^{c}$ |
| | 500 mg | $0.325\pm0.035^{c}$ | $19.873\pm1.938^{c}$ |
| Positive control | ITA | $0.317\pm0.011^{c}$ | $18.093\pm1.732^{b}$ |
| Negative control | ITD | $0.213\pm0.013^{a}$ | $10.028\pm1.57^{a}$ |
| Normal control | NNT | $0.375\pm0.054^{d}$ | $21.876\pm1.507^{bc}$ |

*ITD: Infected and treated with DMSO, ITA: Infected and treated with Amprocox at 5 mg/kg and NNT: Non infected- non treated. Values are Mean ± SEM of four trials. For the same column, values carrying the same superscript letter are not significantly different at p ≥ 0.05 (Student-Newman-Keuls Test).*

**Table 5. Effect of treatment on total peroxidase in the serum and tissues**

| Plant | Groups and doses | Serum | Intestine |
|---|---|---|---|
| | | Total peroxidase (µmol/min/g of tissue or µmol/min/ml*$10^{-2}$ of serum) | |
| *P. guajava* | 125 mg | $2.453\pm1.214^{a}$ | $0.565\pm0.482^{ab}$ |
| | 250 mg | $2.786\pm0.230^{a}$ | $0.561\pm0.490^{ab}$ |
| | 500 mg | $3.226\pm1.998^{a}$ | $0.770\pm0.067^{b}$ |
| Positive control | ITA | $3.250\pm1.425^{a}$ | $0.729\pm0.553^{b}$ |
| Negative control | ITD | $2.076\pm1.151^{a}$ | $0.366\pm0.114^{a}$ |
| Normal control | NNT | $3.448\pm1.881^{ab}$ | $0.957\pm0.095^{c}$ |

*ITD: Infected and treated with DMSO, ITA: Infected and treated with Amprocox at 5 mg/kg and NNT: Non infected- non treated. Values are Mean ± SEM of four trials.For the same column, values carrying the same superscript letter are not significantly different at p ≥ 0.05 (Student-Newman-Keuls Test).*

**Table 6. Effect of treatment on membrane lipid peroxidation (malondialdehyde) in the serum and tissues**

| Plants | Groups and doses | Serum | Intestine |
|---|---|---|---|
| | | Quantities of malondialdehyde (µM/g of tissue or µM/ml of serum) | |
| *P. guajava* | 125 mg | 0.009±0.000$^{f}$ | 0.029±0.001$^{d}$ |
| | 250 mg | 0.007±0.000$^{d}$ | 0.021±0.004$^{c}$ |
| | 500 mg | 0.005±0.000$^{c}$ | 0.014±0.002$^{b}$ |
| Positive control | ITA | 0.004±0.000$^{b}$ | 0.013±0.004$^{b}$ |
| Negative control | ITD | 0.065±0.005$^{g}$ | 0.160±0.107$^{e}$ |
| Normal control | NNT | 0.001±0.000$^{a}$ | 0.008±0.003$^{a}$ |

*ITD: Infected and treated with DMSO, ITA: Infected and treated with Amprocox at 5 mg/kg and NNT: Non infected- non treated. Values are Mean ± SEM of four trials. For the same column, values carrying the same superscript letter are not significantly different at p ≥ 0.05 (Student-Newman-Keuls Test).*

**Table 7. Effect of treatment on nitric oxide (µmole/g of tissue or µmole/ml of serum) in the serum and tissues**

| Plant | Groups and doses | Serum | Intestine |
|---|---|---|---|
| | | Quantities of nitric oxide (µmole/g of tissue orµmole/ml of serum) | |
| *P. guajava* | 125 mg | 5.265±0.101$^{b}$ | 0.383±0.036$^{a}$ |
| | 250 mg | 5.609±0.637$^{ab}$ | 0.394±0.024$^{a}$ |
| | 500 mg | 4.734±0.152$^{a}$ | 0.358±0.090$^{a}$ |
| Positive control | ITA | 4.601±1.412$^{ab}$ | 0.342±0.097$^{a}$ |
| Negative control | ITD | 8.743±0.987$^{c}$ | 0.498±0.055$^{b}$ |
| Normal control | NNT | 4.003±0.575$^{a}$ | 0.367±0.027$^{a}$ |

*ITD: Infected and treated with DMSO, ITA: Infected and treated with Amprocox at 5 mg/kg and NNT: Non infected- non treated. Values are Mean ± SEM of four trials. For the same column, values carrying the same superscript letter are not significantly different at p ≥0.05 (Student-Newman-Keuls Test).*

**Table 8. Effect of treatment on reduced GSH (µmole/g of tissue or µmole/ml of serum) in the serum and tissues**

| Plants | Groups and doses | Serum | Intestine |
|---|---|---|---|
| | | Quantities of reduced GSH (µmole/g of tissue or µmole/ml of serum) | |
| *P. guajava* | 125 mg | 0.968±0.66$^{a}$ | 4.003±1.03$^{b}$ |
| | 250 mg | 0.671±0.12$^{a}$ | 1.987±1.91$^{a}$ |
| | 500 mg | 0.675±0.13$^{a}$ | 1.370±0.30$^{a}$ |
| Positive control | ITA | 0.591±0.05$^{a}$ | 1.476±0.61$^{a}$ |
| Negative control | ITD | 1.087±0.57$^{a}$ | 7.049±1.32$^{c}$ |
| Normal control | NNT | 0.599±0.03$^{a}$ | 1.913±0.39$^{a}$ |

*ITD: Infected and treated with DMSO, ITA: Infected and treated with Amprocox at 5 mg/kg and NNT: Non infected- non treated. Values are Mean ± SEM of four trials. For the same column, values carrying the same superscript letter are not significantly different at p ≥0.05 (Student-Newman-Keuls Test).*

**Table 9. Effect of treatment on SOD (µmole/g of tissue or µmole/ml of serum) in the serum and tissues**

| Plants | Groups and doses | Serum | Intestine |
|---|---|---|---|
| | | Quantities of SOD (µmole/g of tissue or µmole/ml of serum) | |
| *P. guajava* | 125 mg | 0.979±0.02$^{cd}$ | 3.166±0.80$^{a}$ |
| | 250 mg | 0.821±0.07$^{b}$ | 2.475±0.81$^{a}$ |
| | 500 mg | 0.597±0.40$^{a}$ | 2.116±0.20$^{a}$ |
| Positive control | ITA | 0.999±0.00$^{d}$ | 1.913±0.39$^{a}$ |
| Negative control | ITD | 1.190±0.01$^{e}$ | 2.510±0.87$^{a}$ |
| Normal control | NNT | 0.559±0.00$^{a}$ | 2.848±0.81$^{a}$ |

*ITD: Infected and treated with DMSO, ITA: Infected and treated with Amprocox at 5 mg/kg and NNT: Non infected- non treated. Values are Mean ± SEM of four trials. For the same column, values carrying the same superscript letter are not significantly different at p ≥ 0.05 (Student-Newman-Keuls Test).*

### 3.5 Phytochemical Analysis

Phytochemical screening of the most active extracts were consistent with detection of alkaloids, flavonoids, Saponins, Steroids and Tannins, whereas, the absence of polyphenols and terpenoids were noticed (Table 10).

**Table 10. Phytochemical screening of *P. guajava* methanol extract**

| Chemical groups | *P. guajava* |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Polyphenols | - |
| Tannins | + |
| Saponins | + |
| Steroids | + |
| Terpenoids | - |

*+=present, - = Absent*

## 4. DISCUSSION

### 4.1 Anticoccidial Activities

In the present study, higher doses of the methanol extract of *P. guajava* administered have shown good anticoccidial activity in rabbits challenged with *E. intestinalis* by means of improved weight gains, better feed conversion ratio and lower oocyst count. There was a significant reduction in faecal oocyst count with an increase in doses. The reduction in oocyst count probably indicates that *P. guajava* extract impairs development of parasites in the host before the relatively inert oocysts are formed and finally released. However, the lowest OPG was recorded in amprocox treated groups in the experiments indicating the highest prophylactic efficacy among all groups. The results are in full agreement with Conway et al. [19] who studied the effects of different levels of oocysts inocula of *E. acervulina, E. tenella* and *E. maxima* on plasma constituents, packed cell volume, lesion scores and performance in chickens and Elmusharaf et al. [20] who investigated the effect of a Manna-oligosaccharide (MOS) preparation on *E. tenella* infection in broiler chickens. Likewise, Allen et al. [21] reported that the herb *Artemesia annua* reduces oocyst yield. The highest feed conversion ratio observed in the infected untreated rabbits (4.56) provides an evidence of reduction of feed intake due to infection with coccidian oocysts. The highest feed conversion ratio reported in infected rabbits resulted in a significant reduction in body weight. The loss of body weight may be due to excessive loss of body water i.e. diarrhea, which contribute substantially to body weight. Ogbe et al. [22] and Conway et al. [19] also reported a significant reduction in body weight in broilers infected with oocysts of *E. tenella*.

No mortality was observed in un-infected un-medicated control group because no infection was given to the rabbits of this group. While in groups medicated with *P. guajava* extract mortalities were present and the highest mortality (62.5%) was recorded in the infected non-medicated group *P. guajava* extract treated group reduced mortality in dose-dependent manner. The results of the present study are in agreement with Razzaq et al. [23] who observed the highest mortality in infected non-medicated control group. The present findings are also supported by Mpoame et al. [24] who observed 45.5% mortality in infected birds while no mortality occurred in uninfected birds.

### 4.2 Antioxidant Activities

The results of this study showed that the infection led to an imbalance of cytosolic redox status in favor of prooxidants, putting organs in a state of oxidative stress. The increased level of NO in the tissues of negative control animals suggests that macrophages have excessively produced that compound to destroy the *E. intestinalis.*

There was an imbalance between pro-oxidants and antioxidants. Higher NO and MDA levels in infected rabbits are probably due to oxidative stress occurring after coccidial infection. Similar results on oxidative stress in parasitic diseases have been reported by others including [7,21, 25,26]. Wang et al. [26], who for instance, studied the effect of grape seed proanthocyanidin extract on the antioxidant status of chickens infected with *E. tenella* and found that infection (without supplementation) increased NO and MDA but decreased SOD activities. However, grape extract supplementation increased SOD activity but reduced NO and MDA. Administration of methanol extract of *P. guajava* decreased concentrations of NO and MDA, compared to infected rabbits receiving DMSO. This result is similar to the findings of Dkhil et al. [27] who showed that garlic treatment lowered MDA and NO. The increase of the malondialdehyde (MDA) level in organs and serum induced by infection suggests increased membrane peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals.

In this study, groups treated with 125, 250 and 500 mg/kg *P. guajava* extract generally showed significant increase in CAT activity and this could be responsible for the cure effect of the extract. Georgieva et al. [7] showed a decrease in SOD and CAT activities in birds infected with *E. tenella* compared to control birds. In fact, administration of the methanol extract to treated rabbits enhanced catalase and peroxidase profiles, dose-dependently, by acting as a strong free radical quencher and protecting the tissues. Therefore, peroxidase and catalase are essential for the endogenous antioxidative defense system to scavenge reactive oxygen species and maintain the cellular redox balance. CAT and POD decompose hydrogen peroxide and protect tissues from highly reactive hydroxyl radicals. Therefore, reduction in the activity of CAT and POD in negative control animals may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide.

The decrease in GSH levels in animals treated with different extracts might be due to the free radical's neutralizing activities of this extract. They could have reactivated hepatic GSH reductase which is reflected by decreasing the level of lipid peroxidation. Decreased level of GSH is associated with increased lipid peroxidation which is also confirmed in this study. Our observations corroborated the report of Ebokaiwe et al. [28].

The observed decrease in SOD activity following *E. intestinalis* infection and treatment with different doses of plant extract might be due to oxidation of CAT and GSH-Px enzymes. Georgieva et al. [7] showed a decrease in SOD and CAT activities in birds infected with *E. tenella* compared to control birds.

Phenolic compounds (flavones, flavonols, anthocyanins, phenolic acids, etc.) possess a powerful free radical scavenging activity. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction) [29]. Phenolic compounds exhibit antioxidant activity by inactivating free radicals or preventing decomposition of hydroperoxide into free radicals [30]. The results obtained in this study showed that the antiradical scavenging activity was related to the phenolic content.

## 5. CONCLUSION

In accordance to the terms of our research findings, which was to evaluate some pharmacological parameters, therapeutic efficacy and the radical scavenging activities (RSA) of crude methanol extract of *Psidium guajava* against coccidioses. We arrived at the conclusions that, these results therefore provide confirmation to the usage of *Psidium guajava* against coccidioses by Agropastoral farmers in Cameroon. They can be considered as best substitutes to chemical anticoccidials. However, further work is necessary to evaluate the *In vivo* toxicity to further ascertain its therapeutic effect and elucidate the actual mechanism involved in the antioxidant activity of this plant.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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