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In vitro anticoccidial, antioxidant activities and cytotoxicity of *Psidium guajava* extracts.

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Abstract

Background: Coccidiosis remains one of the most important infectious causes of digestive disorders in rabbits. The aim of this study was to evaluate *in vitro* anticoccidial and antioxidant activities of *Psidium guajava* extracts.

Methods: Sporulation inhibition bioassay was used to evaluate the activity of *Psidium guajava* extracts on sporulation of *Eimeria flavescens*, *Eimeria stiedae*, *Eimeria intestinalis* and *Eimeria magna* oocysts and sporozoites. The set up was examined after 24 h and 48 h for the oocysticidal activities and after 12 h and 24 h for anti-sporozoidal activities. The antioxidant activity was determined by measuring FRAP (ferric reducing-antioxidant power), 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and nitric oxide (NO) radical scavenging. The cytotoxicity of the most active extract was determined against animal cell lines fibroblast L929, HEPG2 and Hella cells using MTT assay. The impact of the toxicity was established by analysing the Selectivity Index (SI) values.

Results: The highest efficacy of tested plant extracts was recorded after 24 h, which varied according to different concentrations of the tested extracts. The highest efficacy was 88.67 ± 2.52% at the concentration of 30 mg/ml of the methanolic extract against *E. intestinalis.* Most extracts including the aqueous extract exhibited good anti-sporozoidal activities against *E. flavescens*, *E. stiedae*, *E. intestinalis* and *E. magna* sporozoites at 1000 µg/ml. The highest viability inhibitory percentage was 97.00 ± 1.73% at a concentration of 1000 µg/ml of *P. guajava* methanolic extract against *E. intestinalis* sporozoites. These results also showed that methanolic and Ethyl Acetate extract, possessed strong antioxidant activities (IC50<20 µg/ml). The methanolic extract of *P. guajava* exhibited CC_{50} of>30 µg/ml against selected cell lines, suggesting that the compounds are not toxic. Phytochemical screening of the most active extract showed presence of alkaloids, flavonoids, saponins and phenols.

Conclusion: These results provide confirmation to the usage of *Psidium guajava* against coccidioses by Agricultural farmers in Cameroon.

Keywords: *Psidium guajava*, Anticoccidial activity, Antioxidant, Eimeria species, Cameroon.

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Introduction

In recent years, there has been increasing commercial production of rabbits as a source of protein. The consumers prefer rabbits for their low cholesterol and fat contents and high levels of essential amino-acid [1]. In addition to this commercial value, these animals are used as very important models for medical research and as pets [2]. Therefore, rabbit production become one of the important animal resources in the world [1]. However, coccidiosis remains one of the most important infectious causes of digestive disorders in rabbits [3]. According to a recent estimate [4], coccidiosis may cost the US rabbit industry about $127 million annually and likewise similar losses may occur worldwide.

Coccidiosis is caused by intracellular protozoon parasites of the genus *Eimeria* and causes significant mortality in domestic rabbits. Coccidiosis is one of the most frequent and prevalent parasitic diseases, accompanied by weight loss, mild intermittent to severe diarrhea with faeces containing mucus or blood and results in dehydration, decreased rabbit breeding [5]. The disease is seen most often in rearing establishments where sanitation is poor. So far, 15 species of *Eimeria* in rabbits have been identified [6]. Today 14 species of Eimeria are known to infect the intestine while one is located in the biliary duct of the liver. Two types of coccidiosis, intestinal and hepatic are described in rabbits. The intestinal coccidial species which cause weight reduction, diarrhoea and mortality due to villi atrophy leads to malabsorption of nutrients, electrolyte imbalance, anaemia, hypoproteinemia and dehydration [7]. The rabbit intestinal coccidia parasitize distinct parts of the intestine and at different depths of the mucosa [8]. Thus coccidiosis is probably the most expensive and wide spread infectious disease in commercial rabbit systems.

Most of the current anti-coccidial drugs show low efficacy and cause deleterious side effects. The extensive use of chemical anti-coccidial drugs in controlling this disease has led to the development of drug-resistant parasites [9]. Parasite resistance and the side effects of some of the anti-coccidial drugs have serious consequences on disease control. In the surrounding environment, commonly used disinfectants include some phenolic products such as ammonia, methyl bromide and carbon disulfide. Toxic effects of these products represent a danger to the staff and health of animals and therefore their use has been restricted [10]. Because of widespread drug resistance constraints [11], residual effects of drugs in meat of animals and toxic effects of disinfectants, scientists all over the world are shifting towards alternative approaches for the control of parasitic problems [12].

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 [13], which plays an important role in defending against parasitic infections.

Another free radical oxidative species, nitric oxide promotes vasodilation and hemorrhage in coccidian infections which could be toxic to both parasites as well as to host cells harboring the coccidian parasite [14].

Georgieva et al. [15] observed that *E. acervulina* oocytes 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 [16]. Naidoo et al. [17] also described antioxidant rich plant extracts as potential candidates in controlling coccidiosis in poultry. Therefore, the use of natural antioxidants may alleviate 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 been reported that *Psidium guajava* leaf extract has a wide spectrum of biological activities such as anticough, antibacterial, haemostasis [18,19], antidiarrhoeal narcotic [20], and antioxidant properties [21]. This work was therefore aimed at evaluating the anticoccidial and antioxidant activities of crude extracts of *P. guajava* in order to justify its usage by Agricultural farmers as an anticoccidial drug.

Materials and Methods

Plant material

The 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.

Preparation of extract

Methanol, hexane and Ethyl Acetate extracts were obtained using the procedure described by Wabo Poné et al. [22]. Briefly, 100 g of stored powder were macerated in 1.5 L of each of the organic solvents. This helped to remove the principal natural compounds of the plants [23]. The mixture was stirred daily and 72 h later, these solutions were then filtered using Whatman Paper N 3. The filtrate was concentrated by evaporating the solvent at 75°C using a rotatory evaporator (Buchi R-200) to obtain the extracts.

For the aqueous extract (Infusion), a similar procedure was carried out except for the fact that distilled water was heated at 100°C and 100 g of the stored powder were poured into 1.5 L of hot distilled water. The mixture was stirred and the solution filtered using a tea sieve and filter paper. The methanolic, hexane, Ethyl Acetate and aqueous extracts obtained were kept in a refrigerator at 4ºC for further processing.

Anticoccidial activities of the extracts

Preparation of culture media

Dichromate ($K_2Cr_2O_7$) Potassium: 2.5% Potassium dichromate were prepared by dissolving 2.5 g of potassium dichromate in 100 ml of distilled water. This culture medium was stored and used to prepare our plant extract concentrations.

Preparation of hanks buffered salt solution (HBSS):

Buffer HBSS: KCl	0.4 g
KH_2PO_4	.0.06 g
NaCl	8.0 g
$NaHCO_3$	0.35 g
Na_2HPO_4	0.048 g
D-glucose	1.0 g

Water was added up to 1L and the buffer frozen for storage

Preparation of the excystation solution: 125 ml of HBSS were added to 0.32 g of trypsin, 0.25 g Bile Salt and 0.3 g of taurocholate and the pH was adjusted to 7.6 using NaOH.

Preparation of sporulated oocysts: Field Isolates of *Eimeria flavescens* oocysts were collected from the large intestine while occysts of *E. stiedae* were collected from the gall bladders and necrotic hepatic lesions of naturally infected rabbits. These oocysts were washed and concentrated by the flotation method [24]. The sporulated oocysts were stored in 2.5% potassium dichromate at 4°C until they were used for experimental infections. *Eimeria intestinalis* and *Eimeria magna* were kindly provided by Alisson Niepceron (INRA, BASE, Tours, France). The *Eimeria flavescens*, *Eimeria intestinalis*, *Eimeria magna* and *E. stiedae* field isolates were maintained by periodic passage through young Rabbits in the Laboratory of Biology and Applied Ecology.

Preparation of stock solutions: For the aqueous extracts, 1200 mg of each extract were weighed using an electric scale balance and then 20 ml of distilled water introduced into the mortar. After homogenization, the mixture was transferred into a beaker. For the organic extract, a stock solution was equally prepared and the same amount of dry extract was first mixed with 0.3 ml of Dimethyl sulfoxide (DMSO) to facilitate dissociation of the organic extract with water. Stock solutions with a concentration of 40 mg/ml were thus obtained. By successive dilutions, we obtained solutions of concentration 40, 20, 10 and 5 mg/ml for the oocysticidal evaluation. For the anti sporozoidal evaluation, a working stock solution of 2000 µg/ml of the plant extract solution was prepared by weighing 20 mg of crude extract and dissolving it in 10 ml of distilled water. This was well mixed and serial dilution was carried out to obtain solutions of concentration 1500, 1000, 500, 250 µg/ml.

***In vitro* oocysticidal effect of extracts:** Petri dishes were used to evaluate *in vitro* disinfectant activities. Each well contained a total volume of 2 ml of each concentration of the extracts (2.5, 5, 10, 20 and 30 mg/ml) inoculated with equal number of unsporulated oocysts and incubated at 28°C. For comparison, phenol was used as the reference disinfectant. The set up was examined after 24 h and 48 h. The number of sporulated and non-sporulated oocysts were counted and the percentage of sporulation was estimated by counting the number of sporulated oocysts in a total of 100 oocysts. The sporulation inhibitory percentage was calculated as follows.

$$Sporulation\ (sp)\ inhibition\ percentage\ (\%) = \frac{Sp\ \%\ of\ control - Sp\ \%\ of\ extract}{Sp\ \%\ of\ control} \times 100$$

***In vitro* anti-sporozoidal effect of extracts:** Stored oocysts in $K_2Cr_2O_7$ were washed several times with HBSS (pH 7.2) until the $K_2Cr_2O_7$ was completely removed. The oocysts were then incubated in a water bath at 41°C and shaken during incubation for 60 min. The suspension was centrifuged at 3,000 – 5,000 x g 10 min and resuspended in HBSS. Liberated sporozoites were washed with HBSS. The sporozoites were counted using the malassez counting chamber.

Petri dishes were used to evaluate the *in vitro* sporocidal activities. Each well contained a total volume of 2 ml of each concentration of the extracts (125, 250, 500, 750 and 1000 µg/ml) and inoculated with equal number of sporozoites. For comparison, amprocox was used as the reference drug. The set up was examined after 12 h and 24 h. The number of viable and non-viable sporozoites were counted and the percentage of viability was estimated by counting the number of viable sporozoites in a total of 100 sporozoites.

The viability inhibitory percentage was calculated as follows.

$$Viability\ (Vi)\ inhibition\ percentage\ (\%) = \frac{Vi\ \%\ of\ control - Vi\ \%\ of\ extract}{Vi\ \%\ of\ control} \times 100$$

Antioxidant activities

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay: The radical scavenging activities of crude extracts were evaluated spectrophotometrically using the 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical [25]. When DPPH reacts with an antioxidant compound which can donate hydrogen, it is reduced. The changes in color were measured at 517 nm under UV/Visible light spectrophotometer (Jenway, Model 1605). Pure methanol was used to calibrate the counter. The extract (2000 µg/mL) was twofold serially diluted with methanol. One hundred microliters of the diluted extract were mixed with 900 µL of 0.3 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanol solution, to give a final extract concentration range of 12.5 - 200 µg/mL (12.5, 25, 50, 100 and 200 µg/mL). After 30 min of incubation in the dark at room temperature, the optical densities were measured at 517 nm. Ascorbic acid (Vitamin C) was used as control. Each assay was done in triplicate and the results, recorded as the mean ± standard deviation (SD) of the three findings, were presented in tabular form. The radical scavenging activity (RSA, in %) was calculated as follows:

$$RSA = \frac{Absorbance\ of\ DPPH\ -\ Absorbance\ of\ sample}{Absorbance\ of\ DPPH} \times 100$$

The radical scavenging percentages were plotted against the logarithmic values of concentration of test samples and a linear regression curve was established in order to calculate the RSA50 or IC50 which is the concentration of the sample necessary to decrease by 50% the total free DPPH radical [26].

Ferric reducing/antioxidant power (FRAP) assay: The ferric reducing power was determined by the Fe^{3+} - Fe^{2+} transformation in the presence of the extracts. The Fe^{2+} was monitored by measuring the formation of Perl's Prussian blue at 700 nm. Different volumes (400, 200, 100, 50, 25 µL) of methanolic extracts prepared at 2090 µg/mL were mixed with 500 µL of phosphate buffer (pH 6.6) and 500 µL of 1% potassium ferricyanide and incubated at 50°C for 20 min. Then 500 µL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant (500 µL) was diluted with 500 µL of water and mixed with 100 µL of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm. All the tests were performed in triplicate and the results were the average of three observations. Vitamin C was used as a positive control. Increased absorbance of the reaction mixture indicated a higher reduction capacity of the sample [27].

Nitric oxide radical scavenging (NO) assay: The method reported by Chanda and Dave [28] was used with slight modification. To 0.75 mL of 10 mM sodium nitroprusside in phosphate buffer was added 0.5 mL of extract or reference compounds (Vitamin C and Butylated hydroxytoluene (BHT)) in different concentrations (62.5 - 1000 µg/mL). The resulting solutions were then incubated at 25°C for 60 min. A similar procedure was repeated with methanol as blank which served as negative control. To 1.25 mL of the incubated sample 1.25 mL of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% N-1-napthylethylenediamine dihydrochloride in water) were added. A final concentration range of 12.5 - 200 µg/mL (12.5, 25, 50, 100 and 200 µg/mL) was obtained. After 5 min of incubation in the dark at room temperature, absorbance of the chromophore formed was measured at 540 nm. Percent inhibition of the nitrite oxide generated was measured by comparing the absorbance values of control and test samples. The percentage of inhibition was calculated according to the following equation:

$$\% \ inhibition = \left(1 - \left(A_1 / A_0\right)\right) \times 100$$

Where, A_1=absorbance of the extract or standard and A_0=absorbance of the negative control.

Total phenol contents (TPC): The amount of total phenols was determined by Folin-Ciocateu Reagent method. The reaction mixture consisted of 20 µL of extract (2000 µg/mL), 1380 µL of distilled water, 200 µl of 2N FCR (Folin Ciocalteu Reagent) and 400 µL of a 20% sodium carbonate solution. The mixture was incubated at 40°C for 20 min. After cooling, the absorbance was measured at 760 nm. In the control tube, the extract volume was replaced by distilled water. A standard curve was plotted using Gallic acid (0-0.2 µg/mL). The tests were performed in triplicate and the results expressed as milligrams of Gallic Acid Equivalents (mgGAE) per gram of extract.

Total flavonoid content (TFC): The amount of total flavonoids was determined by the Aluminum chloride method. Methanolic solution of extracts (100 µL, 2000 µg/ml) was mixed with 1.49 mL of distilled water and 30 µL of a 5% $NaNO_2$ solution. After 5 min, 30 µL of 10% $AlCl_3H_2O$ solution were added. After 6 min, 200 µl of 0.1 M sodium hydroxide and 240 µl of distilled water were added. The solution was well mixed and the increase in absorbance was measured at 510 nm using a UV-Visible spectrophotometer. Total flavonoid content was calculated using the standard catechin calibration curve. The results were expressed as milligrams of Catechin Equivalents (mgCE) per gram of extract.

Evaluation of plant extracts cytotoxicity

The cytotoxicity of the most active extract was evaluated on animal cell lines fibroblast L929, HEPG2 and Hella cells using MTT assay as described by Mosmann [29]. Briefly, cells (10^4 cells/200 ml/well) were seeded into 96-well flat-bottom tissue culture plates in complete medium (10% foetal bovine serum, 0.21% Sodium Bicarbonate (Sigma, USA) and 50 mg/ml gentamicin). After 24 h, plant extracts at different concentrations were added and plates incubated for 48 h in a humidified atmosphere at 37ºC and 5% CO_2. 10% DMSO (v/v) was used as a positive inhibitor. Thereafter, 20 µl of a stock solution of MTT (5 mg/mL in 1X phosphate buffered saline) were added to each well, gently mixed and each plate incubated for another 4 h. After spinning the plates at 1500 rpm for 5 min, supernatants were removed and 100 ml of 10% DMSO were added in each well to stop the reaction of extracts. Formation of formazon obtained after transformation of tetrazolium was read on a microtiter plate reader at 570 nm. The 50% cytotoxic concentration (CC_{50}) of plant extract was determined by analysis of dose–response curves, according to the cytotoxicity gradient of plant extracts established by Malebo et al. [30]. Also, the Selectivity Index (SI) was calculated using the following formula:

$$SI = \frac{CC50}{CC50}$$

Phytochemical screening

The most active extract was tested for the presence of phenolic compounds, alkaloids, flavonoids, Polyphenols, tannins, saponin, triterpenes and steroids using standard procedures described by Builders et al. [31].

Statistical analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA) and presented as mean ± standard deviation (SD) of three replications. The levels of significance, considered at $P<0.05$, were determined by Waller-Duncan test using the Statistical Package for Social Sciences (SPSS) software version 12.0.

Results and Discussion

Results

Anticoccidial activities

***In vitro* oocysticidal activities of *P. guajava* extracts:** The *in vitro* oocysticidal activity of different extracts from the plants against *Eimeria intestinalis*, *Eimeria magna, Eimeria flavescens and Eimeria stedai* strains is summarized in (Table 1). It can be seen from Table 1 that about 90% of oocysts of *Eimeria sp* managed to sporulate in the control incubations containing oocysts and DMSO or $K_2Cr_2O_7$. The highest efficacy of tested plant extracts was recorded after 24 h. post exposure which varied according to different concentrations of the tested extracts. Concerning *P. guajava* extracts, the highest efficacy was 88.67 ± 2.52% at the concentration of 30 mg/ml of methanolic extracts against *Eimeria intestinalis*. On the contrary the lowest efficacy was 7.00 ± 4.36% at the concentration of 2.5 mg/ml of the hot water extract on *Eimeria flavescens* after 48 h of incubation. Passing through the other used concentrations of *P. guajava* extracts (2.5, 5, 10 and 20 mg/ ml), they showed reduced efficacy depending on species of *Eimeria* tested.

***In vitro* anti-sporozoidal activities of *P. guajava* extracts:** Different concentrations of *P. guajava extracts* showed concentration dependent inhibition for viability of coccidial sporozoites of different *Eimeria* species as compared to control groups Control-I (DMSO) and Control-II (HBSS) as shown in (Table 2). According to our results, most extracts including aqueous extracts exhibited good antisporozoidal activities against *E. flavescens*, *E. stiedae*, *E. intestinalis* and *E. magna* strains at 1000 µg/ml. The highest viability inhibitory percentage was 97.00 ± 1.73% at a concentration of 1000 µg/ml of *P. guajava* methanolic extract against *E. intestinalis* strain (Table 2). The lowest efficacy was 8.67 ± 2.08% at a concentration of 125 µg/ml of the infusion extract against *E. magna*.

In vitro Antioxidant activities of P. guajava extracts

Effects of *P. guajava* extracts on the DPPH radical: The DPPH radical scavenging activity of different extracts of *P. guajava* was evaluated and the results are shown in (Table 3). All the extracts of *P. guajava* exhibited stronger antioxidant activities, compared to that of the standard antioxidant molecule (Vitamin C) used. The hot water extract showed the lowest activity at any concentrations with an inhibition percentage of 70.52% at 200 µg/ml, while the methanolic extract showed the highest activity (94.59%) at the concentration 200 µg/ml. However, there was no significant ($p>0.05$) difference between the activity of Vitamin C and that of the methanolic and ethylacetate extracts of *P. guajava* at the concentration 200 µg/ml.

Table 1. *Sporulation inhibition percentage of P. guajava extracts on different Eimeria strains.*

Conc mg/ml	Extract	Incubation time and *Eimeria* strains							
		24 h				48 h			
		E. intestinalis	*E. magna*	*E.flavescens*	*E. stedai*	*E. intestinalis*	*E. magna*	*E. flavescens*	*E. stedai*
2.5	IF	17.00 ± 11.53^{ab}	9.00 ± 2.65^{a}	7.67 ± 3.06^{a}	19.67 ± 3.10^{a}	9.00 ± 5,57^{a}	8.00 ± 3.61^{a}	7.00 ± 4.36^{a}	16.00 ± 1.73^{a}
	HE	13.33 ± 1.16^{a}	18.00 ± 3.00^{b}	10.33 ± 2.08^{b}	11.00 ± 2.65^{ab}	12.33 ± 1.16^{a}	16.67 ± 1.53^{b}	9.67 ± 1.53^{a}	7.00 ± 3.61^{a}
	EA	21.67 ± 2.52^{ab}	20.00 ± 2.00^{b}	27.00 ± 6.56^{c}	21.33 ± 1.53^{ab}	20.33 ± 3.06^{b}	18.67 ± 2.52^{b}	25.67 ± 6.03^{b}	18.33 ± 3.06^{a}
	ME	31.33 ± 4.16^{b}	21.67 ± 1.53^{b}	27.00 ± 6.56^{d}	23.67 ± 1.53^{b}	23.67 ± 2.89^{b}	20.00 ± 1.00^{b}	25.67 ± 6.03^{b}	19.00 ± 1.00^{a}
5	IF	15.00 ± 1.00^{a}	11.67 ± 2.52^{a}	13.33 ± 1.53^{a}	25.33 ± 3.22^{a}	12.33 ± 1.53^{a}	10.00 ± 3.00^{a}	12.00 ± 1.00^{a}	21.00 ± 2.65^{a}
	HE	36.67 ± 3.22^{b}	23.33 ± 3.21^{b}	31.00 ± 6.56^{b}	13.67 ± 2.52^{b}	34.67 ± 3.22^{b}	22.00 ± 2.65^{b}	29.33 ± 6.66^{b}	9.00 ± 3.00^{b}
	EA	38.33 ± 4.04^{b}	28.33 ± 2.08^{b}	48.33 ± 6.35^{b}	30.33 ± 2.08^{c}	37.33 ± 3.51^{b}	26.33 ± 3.06^{b}	47.67 ± 6.66^{b}	25.33 ± 3.06^{b}
	ME	53.33 ± 6.11^{c}	38.33 ± 5.51^{c}	48.33 ± 6.35^{c}	39.67 ± 4.51^{d}	47.67 ± 8.51^{c}	36.67 ± 5.51^{c}	47.67 ± 6.66^{c}	36.33 ± 6.51^{c}
10	IF	38.00 ± 4.00^{a}	26.00 ± 4.00^{a}	31.00 ± 2.00^{a}	38.00 ± 4.36^{a}	35.67 ± 4.51^{a}	24.33 ± 4.04^{a}	29.33 ± 2.08^{a}	34.67 ± 4.04^{a}
	HE	47.00 ± 4.58^{b}	36.33 ± 4.16^{b}	40.67 ± 1.53^{b}	27.33 ± 3.06^{b}	45.67 ± 4.51^{b}	35.00 ± 4.58^{ab}	39.33 ± 2.08^{b}	24.00 ± 5.00^{ab}
	EA	46.00 ± 2.00^{b}	39.67 ± 2.52^{b}	50.67 ± 1.53^{b}	41.67 ± 2.52^{c}	44.00 ± 2.65^{b}	38.00 ± 2.00^{b}	50.33 ± 0.58^{b}	37.00 ± 2.00^{ab}
	ME	51.33 ± 2.52^{b}	47.33 ± 1.53^{c}	50.67 ± 1.53^{c}	48.33 ± 3.06^{c}	48.67 ± 2.08^{b}	45.00 ± 1.73^{c}	50.33 ± 0.58^{c}	45.33 ± 0.58^{b}
20	IF	56.33 ± 6.66^{a}	42.00 ± 4.00^{a}	53.67 ± 8.02^{a}	49.00 ± 2.00^{a}	55.67 ± 6.81^{a}	41.00 ± 5.00^{a}	52.00 ± 7.55^{a}	45.00 ± 4.00^{a}
	HE	59.33 ± 9.07^{a}	47.33 ± 2.56^{a}	57.00 ± 4.58^{a}	44.00 ± 4.00^{a}	57.00 ± 6.58^{a}	45.67 ± 3.51^{ab}	55.67 ± 4.73^{a}	40.00 ± 5.00^{a}
	EA	67.00 ± 3.00^{ab}	54.33 ± 2.52^{b}	71.00 ± 1.00^{ab}	56.00 ± 2.65^{a}	65.00 ± 2.65^{ab}	52.67 ± 3.51^{b}	69.67 ± 1.16^{ab}	52.00 ± 3.61^{a}
	ME	73.33 ± 2.52^{b}	67.00 ± 2.65^{c}	71.00 ± 1.00^{b}	68.67 ± 3.22^{a}	71.67 ± 2.08^{b}	66.00 ± 2.65^{c}	69.67 ± 1.16^{b}	65.33 ± 2.08^{b}
30	IF	69.67 ± 6.51^{a}	56.67 ± 4.16^{a}	65.67 ± 6.66^{a}	64.33 ± 3.51^{a}	68.67 ± 6.51^{a}	55.67 ± 3.21^{a}	64.33 ± 6.51^{a}	62.00 ± 4.36^{a}
	HE	51.33 ± 38.42^{a}	63.33 ± 3.79^{ab}	68.00 ± 4.59^{a}	58.33 ± 4.04^{a}	71.67 ± 3.79^{a}	62.00 ± 4.00^{ab}	67.33 ± 4.04^{a}	55.00 ± 3.46^{a}
	EA	77.00 ± 1.00^{a}	68.33 ± 3.79^{b}	80.67 ± 2.52^{ab}	70.00 ± 4.36^{a}	75.67 ± 4.16^{a}	67.00 ± 4.36^{b}	79.67 ± 1.53^{ab}	64.67 ± 3.79^{a}
	ME	90.00 ± 1.73^{a}	76.00 ± 3.00^{c}	80.67 ± 2.52^{b}	78.00 ± 3.00^{b}	88.67 ± 2.52^{b}	76.00 ± 1.00^{c}	79.67 ± 1.53^{b}	75.00 ± 1.00^{b}
Negative Control	DMSO +$K_2Cr_2O_7$	8.00 ± 3.61	8.00 ± 2.00	8.00 ± 1.00	8.33 ± 0.58	5.33 ± 2.08	6.33 ± 1.53	6.67 ± 0.58	6.33 ± 0.58
	$K_2Cr_2O_7$	10.33 ± 2.10	9.33 ± 1.53	10.33 ± 1.53	10.33 ± 0.58	8.67 ± 1.53	8.00 ± 1.73	8.33 ± 1.52	9.00 ± 1.00
Positive Control	5%	100.00 ± 0.00	100.00 ± 0.00	100 ± 0.00	100.00 ± 0.00	86.67 ± 10.69	86.67 ± 10.69	84.00 ± 1.00	82.00 ± 1.00

ME: Methanolic extract, HE: Hexane extract, EAE: Ethyl acetate extract, IF: Infusion extract, DMSO: Diméthylsulfoxide and $K_2Cr_2O_7$: Potassium dichromate. The results are the mean ± SD of triplicate tests evaluated after 24 and 48 h of incubation at room temperature. For the same column same concentrations, values carrying the same superscript letter are not significantly different at $p \geq 0.05$ (Student-Newman-Keuls test).

The concentrations which inhibited 50% of DPPH (IC_{50}) are presented in (Table 3). These results show that the hot water extract had a high IC_{50} (low activity). The ethyl acetate and the methanol extract of *P. guajava* had the lowest IC_{50} (i.e. had the highest activity). The methanol extract of *P. guajava* had the lowest IC_{50} (i.e. the highest activity).

Ferric reducing/antioxidant power (FRAP) of *P. guajava* extracts: The reducing power was determined by the Fe^{3+}- Fe^{2+} transformation in the presence of the extracts of *P. guajava*, and the results obtained are shown in (Table 4). The hot water extract showed the lowest reducing power while the standard (Vitamin C) exhibited the highest reducing power at the concentrations of 100 and 200 µg/ml. At 100 µg/ml, there was no significant difference between the reducing power of Vitamin C (2,510 ± 0,65) and the methanolic extract of *P. guajava* (2,517 ± 0,01). However, the hot water extract showed the lowest optical densities (i.e. lowest reducing power) at every concentration. The remaining extracts exhibited varied activities from one extract to another at each concentration.

Effects of *P. guajava* extracts on Nitric oxide: The results of the scavenging capacity against nitric oxide were recorded in terms of percentage inhibition as presented in (Table 5). The extracts of *P. guajava* showed considerable antioxidant potential. The methanolic and ethylacetate extracts revealed the highest percentage inhibition indicating the best nitric oxide scavenging activity. However, hexane extracts of *P. guajava* showed the lowest scavenging activity at every concentration.

Total phenolic content of *P. guajava* extracts: The total phenolic content of *P. guajava* extracts were determined in this study using Folin-Ciocateu Reagent method and the results are presented in (Table 6). The concentration of phenolic compounds in the methanolic extract (18,536 mgGAE/mg) was higher than in all other extracts. The methanolic and Ethyl Acetate had relatively the same concentration (p>0.05) and the lowest concentration of phenolic compounds was observed in the infusion extract (8.380 mgGAE/mg).

Total flavonoid content of *P. guajava* extracts: The total flavonoid contents of the various extracts are presented in (Table 6). The result obtained showed that the methanol extract had the highest flavonoid content (1,991 mgCE/mg) while the infusion extract showed the lowest value of flavonoid content.

Cytotoxicity test: In order to evaluate the cytotoxicity effect, L929, HEPG2 and Hella cells were exposed to *P. guajava* methanolic extract, for 48 h and cell grown inhibition was accessed using MTT assay. In our current study, the methanolic extract exhibited CC_{50} of >30 µg/ml against (Table 7) the selected cell lines, suggesting that the compounds are not toxic.

Selectivity index: The selectivity index of the methanolic extract was then evaluated using the MTT assay on L929, HEPG2 and Hella cells in order to check that their toxicity was specific to the parasite (Table 7). The impact of toxicity was established by analysing the selectivity index (SI) values. In our study, selectivity index values for the tested extract ranged

Table 2. *Viability inhibitory percentage of P. guajava extracts on different Eimeria strains.*

Conc µg/ml	Extract	Incubation time and *Eimeria* strains							
		12 h				24 h			
		E. **intestinalis**	***E. magna***	***E. flavescens***	***E. stedai***	***E.*** **intestinalis**	***E. magna***	***E. flavescens***	***E. stedai***
125	IF	7.67 ± 3.06^{a}	15.00 ± 2.65^{a}	3.00 ± 4.36^{a}	13.00 ± 1.73^{a}	24.00 ± 11.53^{a}	8.67 ± 2.08^{a}	18.67 ± 3.06^{a}	24.67 ± 3.06^{a}
	HE	9.33 ± 1.15^{a}	24.00 ± 3.00^{b}	5.67 ± 1.53^{a}	4.00 ± 3.61^{b}	20.33 ± 1.15^{ab}	9.33 ± 1.15^{a}	21.33 ± 2.08^{a}	16.00 ± 2.65^{b}
	EA	17.33 ± 3.06^{b}	26.00 ± 2.00^{b}	14.00 ± 4.36^{b}	15.33 ± 3.06^{b}	28.67 ± 2.52^{ab}	17.33 ± 3.06^{b}	29.67 ± 4.04^{b}	26.33 ± 1.53^{b}
	ME	20.67 ± 2.89^{b}	27.67 ± 1.53^{b}	21.67 ± 6.03^{b}	16.00 ± 1.00^{b}	38.33 ± 4.16^{b}	20.67 ± 2.89^{b}	38.00 ± 6.56^{c}	28.67 ± 1.53^{b}
250	IF	9.33 ± 1.53^{a}	17.67 ± 2.52^{a}	8.00 ± 1.00^{a}	18.00 ± 2.65^{a}	22.00 ± 1.00^{a}	9.33 ± 1.53^{a}	24.33 ± 1.53^{a}	30.33 ± 3.21^{a}
	HE	31.67 ± 3.21^{b}	29.33 ± 3.21^{b}	25.33 ± 6.66^{b}	6.00 ± 3.00^{b}	43.67 ± 3.21^{b}	31.67 ± 3.21^{b}	42.00 ± 6.56^{b}	18.67 ± 2.52^{b}
	EA	34.33 ± 3.51^{b}	34.33 ± 2.08^{b}	32.00 ± 3.61^{b}	22.33 ± 3.06^{b}	45.33 ± 4.04^{b}	34.33 ± 3.51^{b}	47.67 ± 4.51^{b}	35.33 ± 2.08^{b}
	ME	44.67 ± 8.50^{c}	44.33 ± 5.51^{c}	43.67 ± 6.66^{c}	33.33 ± 6.51^{c}	60.33 ± 6.11^{c}	44.67 ± 8.50^{c}	59.33 ± 6.35^{c}	44.67 ± 4.51^{c}
500	IF	32.67 ± 4.51^{a}	32.00 ± 4.00^{a}	25.33 ± 2.08^{a}	31.67 ± 4.04^{a}	45.00 ± 4.00^{a}	32.67 ± 4.51^{a}	42.00 ± 2.00^{a}	43.00 ± 4.36^{a}
	HE	42.67 ± 4.51^{b}	42.33 ± 4.16^{b}	35.33 ± 2.08^{b}	21.00 ± 5.00^{b}	54.00 ± 4.58^{b}	42.67 ± 4.51^{b}	51.67 ± 1.53^{b}	32.33 ± 3.06^{b}
	EA	41.00 ± 2.61^{b}	45.67 ± 2.52^{b}	38.33 ± 4.16^{b}	34.00 ± 2.00^{b}	53.00 ± 2.00^{b}	41.00 ± 2.65^{b}	55.00 ± 3.61^{b}	46.67 ± 2.52^{b}
	ME	45.67 ± 2.08^{b}	53.33 ± 1.53^{c}	46.33 ± 0.58^{c}	42.33 ± 0.58^{c}	58.33 ± 2.52^{b}	45.67 ± 2.08^{b}	61.67 ± 1.53^{c}	53.33 ± 3.06^{c}
750	IF	52.67 ± 6.81^{a}	48.00 ± 4.00^{a}	48.00 ± 7.55^{a}	42.00 ± 4.00^{a}	63.33 ± 6.66^{a}	52.67 ± 6.81^{a}	64.67 ± 8.02^{a}	54.00 ± 2.00^{a}
	HE	54.00 ± 6.56^{a}	53.33 ± 2.52^{a}	51.67 ± 4.73^{a}	37.00 ± 5.00^{ab}	66.33 ± 9.07^{a}	54.00 ± 6.56^{a}	68.00 ± 4.58^{a}	49.00 ± 4.00^{a}
	EA	62.00 ± 2.65^{ab}	60.33 ± 2.52^{b}	57.33 ± 1.53^{ab}	49.00 ± 3.61^{b}	74.00 ± 3.00^{ab}	62.00 ± 2.65^{ab}	74.33 ± 1.53^{ab}	61.00 ± 2.65^{b}
	ME	68.67 ± 2.08^{b}	73.00 ± 2.65^{c}	65.67 ± 1.15^{b}	62.33 ± 2.08^{c}	80.33 ± 2.52^{b}	68.67 ± 2.08^{b}	82.00 ± 1.00^{b}	73.67 ± 3.21^{c}
1000	IF	65.67 ± 6.51^{a}	62.67 ± 4.16^{a}	60.33 ± 6.51^{a}	59.00 ± 4.36^{a}	76.67 ± 6.51^{a}	65.67 ± 6.51^{a}	76.67 ± 6.66^{a}	69.33 ± 3.51^{a}
	HE	68.67 ± 3.79^{a}	69.33 ± 3.79^{ab}	63.33 ± 4.04^{a}	52.00 ± 3.46^{b}	58.33 ± 38.42^{a}	68.67 ± 3.79^{a}	79.00 ± 4.58^{a}	63.33 ± 4.04^{ab}
	EA	72.67 ± 4.16^{a}	74.33 ± 3.79^{b}	69.33 ± 3.22^{ab}	61.67 ± 3.79^{b}	84.00 ± 1.00^{a}	72.67 ± 4.16^{a}	86.00 ± 3.00^{ab}	75.00 ± 4.36^{b}
	ME	85.67 ± 2.52^{b}	82.00 ± 3.00^{c}	75.67 ± 1.53^{b}	72.00 ± 1.00^{c}	97.00 ± 1.73^{a}	85.67 ± 2.52^{b}	91.67 ± 2.52^{b}	83.00 ± 3.00^{c}
Negative Control	DMSO	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
	HBSS	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
Positive Control	50µg /ml	79.00 ± 1.00	83.67 ± 10.69	81.00 ± 1.00	78.00 ± 1.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

ME: Methanol extract, HE: Hexane extract, EAE: Ethyl acetate extract, IF: Infusion extract DMSO: Diméthylsulfoxide, HBSS: Buffer Hanks buffered salt solution and $K_2Cr_2O_7$: Potassium dichromate. The results are the mean ± SD of triplicate tests evaluated after 12 and 24 h of incubation at room temperature. For the same column same concentrations, values carrying the same superscript letter are not significantly different at $p \geq 0.05$ (Student-Newman-Keuls test).

Table 3. *DPPH radical-scavenging activities of P. guajava.*

Extracts	Concentration of extract (µg/mL) and scavenging activity (%)					IC_{50} (µg/ml)
	12.5	**25**	**50**	**100**	**200**	
IF	42.074 ± 1.42^{bcd}	46.074 ± 0.33^{ab}	50.370 ± 0.78^{b}	55.555 ± 2.65^{b}	70,518 ± 1,96^{b}	102.831 ± 22.78^{ab}
HE	42.592 ± 3.17^{bcd}	47.037 ± 1.28^{ab}	56.666 ± 1.55^{c}	63.407 ± 4.20^{c}	86,296 ± 3,90^{d}	37.969 ± 13.59^{a}
EA	44.66 ± 1.99^{cd}	70.518 ± 2.11^{cd}	88.518 ± 2.21^{e}	90.296 ± 0.49^{e}	91,925 ± 0,61^{e}	2.879 ± 0.20^{a}
ME	47.185 ± 0.66^{d}	78.740 ± 4.25^{cd}	86.296 ± 4.10^{e}	92.074 ± 1.33^{e}	94,592 ± 0,32^{e}	2.168 ± 0.27^{a}
Vitamin C	76.178 ± 6.69^{e}	86.186 ± 0.62^{e}	87.262 ± 0.75^{e}	90.157 ± 1.03^{e}	93.465 ± 0.37^{e}	1,295 ± 0,14^{a}

For the same column, values carrying the same superscript letter are not significantly different at $p \geq 0.05$ (Student-Newman-Keuls test). ME: Methanolic extract, HE: Hexane extract, EAE: Ethyl acetate extract, IF: Infusion extract.

Table 4. *Ferric reducing power activities of P. guajava extracts.*

Extracts	Concentrations (µg/ml) et absorbance (à 700 nm)				
	12.5	**25**	**50**	**100**	**200**
IF	0.632 ± 0.08^{d}	0.642 ± 0.05^{d}	0.802 ± 0.07^{d}	0.999 ± 0.06^{ab}	1.285 ± 0.06^{b}
HE	0.783 ± 0.03^{e}	0.782 ± 0.03^{e}	0.940 ± 0.03^{d}	1.317 ± 0.03^{b}	1.691 ± 0.02^{c}
EA	0.625 ± 0.06^{d}	1.331 ± 0.04^{f}	1.354 ± 0.04^{f}	1.810 ± 0.02^{c}	2.317 ± 0.07^{e}
ME	1.691 ± 0.07^{g}	1.940 ± 0.03^{h}	2.31 ± 0.03^{h}	2.517 ± 0.05^{d}	2.908 ± 0.07^{g}
Vitamin C	0.028 ± 0.00^{a}	0.044 ± 0.00^{a}	0.056 ± 0.02^{a}	2.510 ± 0.65^{d}	6.339 ± 0.09^{h}

For the same column, values carrying the same superscripts letter are not significantly different at $p \geq 0.05$ (Student-Newman-Keuls test). ME: Methanolic extract, HE: Hexane extract, EAE: Ethyl acetate extract, IF: Infusion extract**.**

between 1.01 to 20.64 µg/ml. The methanolic extract of *P. guajava* showed the highest selectivity index value of 20.64 µg/ml, on L929 cells which was noteworthy as the extracts from this plant showed good anticoccidial activity.

Phytochemical analysis: Phytochemical screening of the most active extracts were consistent with detection of alkaloids, flavonoids, Saponines, Steroids and Tannins, whereas, the absence of polyphenols and terpenoids were noticed (Table 8).

Discussion

In Cameroon as in all developing countries, plants are regularly solicited by farmers to treat recurrent coccidioses. In this study,

Table 5. *Nitric oxide (NO) radical scavenging of P. guajava extracts.*

Extracts	**Concentrations (µg/ml) et pourcentage d'inhibition (%)**				
	12.5	**25**	**50**	**100**	**200**
IF	86.295 ± 0.147^{a}	89.23 ± 0.327^{ab}	89.591 ± 0.269^{ab}	89.634 ± 0.374^{bc}	89.787 ± 0.274^{ab}
HE	81.029 ± 0.211^{a}	81.978 ± 2.037^{a}	84.003 ± 0.546^{ab}	84.349 ± 0.473^{b}	86.738 ± 3.725^{ab}
EA	83.271 ± 4.231^{b}	88.594 ± 0.725^{ab}	89.425 ± 0.798^{ab}	89.627 ± 0.385^{bc}	90.734 ± 0.672^{c}
ME	85.849 ± 1.725^{b}	86.257 ± 0.725^{c}	89.647 ± 0.258^{ab}	88.464 ± 11.151^{bc}	92.349 ± 0.729^{c}
Vitamine C	92.427 ± 3.627^{c}	94.595 ± 2.032^{c}	94.595 ± 1.339^{b}	96.556 ± 0.895^{c}	96.556 ± 0.298^{c}
BHT	94.946 ± 0.800^{c}	96.429 ± 0.110^{d}	97.274 ± 0.526^{c}	97.624 ± 0.027^{d}	99.410 ± 0.055^{d}

For the same column, values carrying the same superscript letter are not significantly different at $p \geq 0.05$ (Student-Newman-Keuls test). ME: Methanolic extract, HE: Hexane extract, EAE: Ethyl acetate extract.

Table 6. *Total phenolic and flavonoid contents of P. guajava extracts.*

Extracts	**Phenols (mgGAE/mg)**	**Flavonoids (mgCE/mg)**
Infusion	8.380 ± 0.80^{bc}	0.494 ± 0.00^{ab}
Hexane	10.461 ± 1.20^{cd}	1.720 ± 0.13^{d}
Ethyl Acetate	15.328 ± 2.13^{ef}	1.881 ± 0.03^{d}
Methanol	18.536 ± 2.17^{f}	1.991 ± 0.18^{d}

Along each column, values with the same superscripts are not significantly different, Waller Duncan (P>0.05).

Table 7. *Selectivity index, CC50 on L929, HEPG2 and Hella cells of P. guajava methanolic extracts.*

Plants	**Cell line**	**CC_{50} (µg/ml)**	**Sporozoidal IC_{50} (µg/ml)**	**Selectivity index (µg/ml)**
P. guajava	L929 cells	148.83	94.99	20.64
	HEPG2 cells	96.24		1.01
	Hella cells	129.29		1.36

Table 8. *Phytochemical screening of P. guajava methanolic extracts.*

Chemical groups/Plant extract	***P. guajava***
Alkaloids	+
Flavonoids	+
Polyphenols	-
Tannins	+
Saponines	+
Steroids	+
Terpenoids	-

we evaluated the anticoccidial and antioxidant activities of crude extracts of one African traditional medicinal plant. The observations that *P. guajava* extract concentrations had an effect on the sporulation of coccidia oocysts indicates that *P. guajava* extracts are able to kill or inhibit growth and development of oocysts. The finding that *P. guajava* had the highest sporulation inhibition at 30 mg/ml suggests that it is more effective in treating coccidiosis. According to our results, most extracts including aqueous extracts exhibited good oocysticidal activity against *Eimeria intestinalis*, *Eimeria magna, Eimeria flavescens* and *Eimeria stedai* strains. The *P. guajava* extract showed maximum sporulation inhibition activity at 30 mg/ml and was observed to be more effective against *Eimeria intestinalis*. Similar to present findings, Molan et al. [32] also observed *in-vitro* sporulation inhibition with aqueous extracts of pine bark (*Pinus radiata*) in three species of avian coccidia. Since extracts have been shown to inhibit endogenous enzyme activities [33], then it is possible that *P. guajava* extract reduced the proportion of sporulation by inhibiting or inactivating the enzymes responsible for the sporulation process as in helminth eggs [34]. Jones et al. [34] suggested that extracts may penetrate the cell wall of oocysts and cause a loss of intracellular components. In the present study, the *P. guajava* extracts might have penetrated the wall of the oocysts and damaged the cytoplasm (sporont) as evidenced by the appearance of abnormal sporocysts in oocysts exposed to higher concentrations. The differences between the four extracts in inhibiting sporulation of coccidia oocysts may be due to differences in chemical composition. The observation that $K_2Cr_2O_7$ could not inhibit sporulation could be explained by the fact that since it is a bactericidal drug as well, it might have killed the bacteria present thereby enhancing the sporulation of oocysts. Potassium dichromate killed bacteria in a sample containing coccidian oocysts thereby enhancing sporulation of coccidia oocysts. Therefore it could be that bacteria if present, could have interfered with the sporulation of oocysts, possibly by competing for nutrients and/or feeding on the oocysts.

The percentage of cells viability under control circumstances (DMSO and HBSS) in this study was comparable with other studies using *Eimeria species* [35,36], therefore the method used may be considered an acceptable model. To our knowledge, this is the first study to evaluate the effects of *P. guajava* as inhibitors of *Eimeria intestinalis*, *Eimeria magna, Eimeria flavescens and Eimeria stedai* sporozoites *in vitro.* Our findings confirm the results of another study on the inhibitory effect of curcumin on the activity of *E. tenella* sporozoites [36]. The mechanism of inhibition is unknown, but may be linked to osmotic effects attributed to extracts [37]. Schubert et al. [38] had demonstrated that extracellular calcium and Ca^{2+} signaling are essential for the invasion of *E. tenella* sporozoites into host cells. Extracts have been shown to activate and desensitize receptors in calcium channels [39]. It is possible that *P. guajava extracts* contribute to the observed inhibition of sporozoite viability by disrupting calcium-mediated signaling in the sporozoites.

The antioxidative profile of various extracts of *P. guajava* is a prelude to finding agent(s) that could be used to reduce oxidative stress associated with coccidioses. Since multiple characteristic reactions and mechanisms are involved in the so-called oxidative stress, using a single test is not sufficient to evaluate the antioxidant potential of plant natural compounds or extracts [40]. Therefore, many antioxidant assays such as DPPH radical scavenging activity, ferric reducing/antioxidant power and nitric oxide scavenging activity methods were chosen in order to evaluate the antioxidant properties of *P. guajava* extracts.

The DPPH assay has been used widely to determine the radical scavenging activity of antioxidant substances [41,42]. The DPPH free radical scavenging activity was significantly ($P<0.05$) higher in the methanol extract followed by Ethyl Acetate; while the infusion and the hexane extracts had the least

DPPH free radical scavenging activity. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen-donating antioxidant due to formation of the non-radical form DPPH-H [43]. The extracts significantly inhibited the activity of DPPH radicals in a dose-dependent manner and the maximum scavenging activities were observed at the concentration of 200 mg per ml. The effect of antioxidants on DPPH radical has been thought to be due to their hydrogen donating ability. Hence, DPPH is usually used as a substrate to evaluate ant oxidative or free radical scavenging activity of antioxidant agents. In our experiment, the high DPPH radical scavenging activities of some extracts were comparable to the standard antioxidant, Vitamin C, suggesting that the extracts have some compounds with high proton donating ability and could therefore serve as free radical inhibitors. However, the organic extract of *P. guajava* demonstrated a more remarkable anti-radical activity with IC50<20 µg/ml. In fact, according to Souri et al. [44], the antioxidant activities of plant extracts are significant when IC50 <20 µg/ml, moderate when 20 µg/ml≤IC50 ≤ 75 µg/ml and weak when IC50>75 µg/ml. There was no significant difference ($p>0.05$) between IC50 values of the organic extracts and ascorbic acid. The higher radical scavenging activity observed in *P. guajava* leaves is perhaps attributed to the higher condensed tannins content in these leaves. In the present study, the condensed tannins content and the radical scavenging activity of *P. guajava* leaves are likely to show a good relationship. Previous studies had also reported the relationship between the high level of polyphenolic compounds and radical scavenging activity [45,46]. On the other hand, the higher DPPH free radical scavenging activity of *P. guajava* extracts may be due to the potential and effective condensed tannins source because of reactions between condensed tannins molecules and radicals resulting in the scavenging of radicals by hydrogen donation [47].

Antioxidants can be reductants, and inactivation of oxidants by reductants can be described as oxido-reduction reactions [48]. The presence of reductants such as antioxidant substances in the samples causes reduction of the ferric to the ferrous form which can be monitored by measuring the formation of Perlis prussian blue at 700 nm. The FRAP assay, therefore, provides a reliable method to study the antioxidant activity of various extracts. In this study, the infusion extracts had moderate reducing power; the highest activity was obtained with the methanol extract and the lowest activity was obtained with the infusion. These data suggest that the extract of *P. guajava* may contain several compounds with intermediate polarity. The methanol extract of *P. guajava* showed significantly ($P<0.05$) higher reducing ability compared to other extracts. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. The methanol extract of *P. guajava* exhibited a higher reducing power. The reducing power of *P. guajava* is mainly correlated to the presence of reductones like ascorbic acid and guava is reported to be rich in ascorbic acid [49]. In the present study we observed a concentration-dependent decrease in the absorbance of the reaction mixture for all the extracts and ascorbic acid. The reducing capacity of extracts is much related to the presence of biologically active compounds (condensed tannins) with potent donating abilities may therefore, serve as an indicator of its potential antioxidant activity [50]. The observed reducing ability of *P. guajava* extracts in the present study could be attributed to the presence of condensed tannins as reported by Omoruyi et al. [51]. Previous studies of Omoruyi et al. [51] and Park and Jhon [52] correlated the reducing power ability of plant extracts to the presence of phenolic content. The antioxidant potential and effectiveness of condensed tannins is generally proportional to the number of hydroxyl (-OH) groups present on the aromatic ring (s) as well as arrangement of the hydroxyl groups and extraction processes.

It is well documented that during chicken coccidiosis, the generation of pro inflammatory mediators, together with the oxidative and Nitrous Oxide (NO) species, contribute principally to inflammatory injury, diarrhea, mortality and weight loss [53]. Therefore, substances that generate oxidative stress or have antioxidant properties such as n-3 fatty acids, g-tocopherol, curcumin, essential oil blends and green tea extracts demonstrated certain coccidiostat effects [54]. It seems that after parasite invasion, free radicals, together with high levels of NO production, are the major factors that compromise the cellular antioxidant defense system. Compounds that are meeting the demands of antioxidant defense system or directly interfere with free radicals, such as tannins, may restore the balance of oxidants/antioxidants, leading to improvement in intestinal integrity and performance during subclinical coccidiosis [17]. Antioxidants act by scavenging the NO radicals [28]. Nitric oxide radical scavenging activity is correlated to the presence of phenolic compounds [55]. There was a significant decrease in the NO radical due to the scavenging ability of extracts and ascorbic acid. The increased nitric oxide radical scavenging activity was observed in every extract of the tested plants. The ethyl acetate extracts showed better scavenging capacity compared to methanolic extract. The nitric oxide scavenging potential may be due to antioxidant principle in the extract which competes with oxygen to react with nitric oxide and thus inhibit the generation of nitrites.

Phenolic compounds exhibit antioxidant activity by inactivating free radicals or preventing decomposition of hydroperoxide into free radicals [56]. Flavonoids' protective effects in biological systems are linked to their ability to transfer electrons to free radicals, chelate metals, activate antioxidant enzymes and reduce radicals of alpha-tocopherol or to inhibit oxidases [56]. The results obtained in this study showed that antiradical scavenging activity was related to the phenolic content. Then, the methanolic crude extract of *P. guajava* was found to have high phenolic contents with 18,536 mgGAE/mg and which may be one of the reasons explaining its high antioxidant activity with an IC50 of 2,168 ± 0,27 (DPPH radical-scavenging activity) and absorbance of 2,908 ± 0,07 at 200 µg/ml (Ferric reducing power activity). There was a positive linear correlation between antioxidant activity index and total phenolic content for all the extracts. These results suggest that the phenolic compounds contribute significantly to the antioxidant capacity of the investigated plant species. In addition, these results are consistent with the findings of many researchers who reported such positive correlation between total phenolic content and antioxidant activity [57]. However, Bajpai et al. [58] disproved the correlation between phenolic compounds and antioxidant activity. The results of antioxidant assays further suggest that these extracts contain powerful free radical scavenging phytochemicals that could be used to fight against free radical upsurge, as well as oxidative stress; and consequently might ameliorate oxidative stress-associated metabolic disorders.

Cytotoxicity screening is the *in vitro* toxicological assessment of specific adverse effects of drugs. Assessment of the cytotoxicity *P. guajava* revealed that the CC_{50} of the methanol extract on L929, HEPG2 and Hella cell lines were above 30 µg/ml indicating the overall safety of *P. guajava.*

According to [30], plants were classified by their cytotoxicity potential as:

(a) high cytotoxicity (CC50<1.0 μg/ml),

(b) moderate (CC_{50} 1.0–10.0 μg/ml)

(c) mild (CC_{50} 10.0–30.0 μg/ml)

(d) nontoxic (CC50>30 μg/ml)

We realize that, the tested extract was found to be non-cytotoxic or with very low toxicity on L929, HEPG2 and Hella mammalian cell lines. It has been reported that *P. guajava* leaf extracts demonstrated no cytotoxicity in clinical trials with humans [59]. In a separate study, Ling et al. [60] reported that some ethanolic extracts including that of *P. guajava* lack cytotoxicity in assays involving 3T3 and 4T1 cells.

Selectivity Index is the ratio of cytotoxicity to biological activity. To estimate the potential of molecules or extracts to inhibit parasite growth without toxicity, Selectivity Index (SI) was introduced. Low SI indicates that the anticoccidial activity is probably due to cytotoxicity rather than activity against the parasite themselves. In contrast, high SI should offer the potential of safer therapy. When a plant extract has a selectivity index value greater than one, it is more active against the target parasite strain and less toxic to the mammalian cells used in the cytotoxicity assay. When its Selectivity Index value is less than one, it is more toxic and less active. In our study the selectivity index values for the tested extracts ranged between 1.01 to 20.64 µg/ml. The methanol extract of *P. guajava* showed the highest Selectivity Index value of 20.64 µg/ml, which was noteworthy as the extracts from this plant showed good anticoccidial activity. This observation may be an indicator of their safety as drugs for mammalian organisms. Our findings, therefore, corroborate the use of *P. guajava* as anticoccidial in Cameroonian folk medicine, and could therefore be inscribed or included in the pharmacopoeia of Cameroon traditional medicine.

Conclusion

Due to widespread development of resistance to anticoccidial drugs, there is shift to reduce the use of these chemical compounds. Efforts have been made to develop new strategies for control of rabbit coccidiosis. These efforts include a search for new agents with anticoccidial activity such as naturally occurring compounds that are considered most effective and safe. The control of oxidative damage caused by Reactive oxygen species and free radicals produced within the cell is a major field of study nowadays. Latest research on natural antioxidants including herbal antioxidants have proved their health benefits against oxidative stress which is involved in the pathology of several diseases in living organisms including coccidiosis in rabbits. They can be considered as best substitutes to chemical anticoccidials. However further experimental studies are required to explore the efficacy of *P. guajava* anticoccidials, antioxidants and their modes of action.

Conflict of Interest Statement

We declare that we have no conflict of interest.

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