



OPEN Antioxidant, anticoccidial, and toxicological evaluation of *Ocimum gratissimum* and *Vernonia amygdalina* leaf extracts against *Eimeria* spp

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The plants *Ocimum gratissimum* and *Vernonia amygdalina* are widely used in traditional medicine for their biological properties, but their antioxidant and anticoccidial activities and toxicological profile remain poorly documented. This study aims to evaluate the antioxidant potential, anticoccidial efficacy against *Eimeria magna* and *Eimeria media*, phytochemical composition, and toxicity of aqueous and hydroacetic extracts from their leaf. Aqueous and hydroacetic leaf extracts of both plants were prepared and quantified for their polyphenol, flavonoid, and tannin contents. Antioxidant activity was determined using the DPPH radical scavenging assay. The anticoccidial activity of graded extract concentrations was evaluated against *Eimeria* oocysts and sporozoites. Toxicity was assessed through lethality tests on *Artemia salina* larvae and clinical evaluations in rats. The extracts of *V. amygdalina* leaf exhibited stronger antioxidant and anticoccidial activities than those of *O. gratissimum*. hydroacetic extracts of both plants showed lower EC₅₀ values (*O. gratissimum*: 51.2 ± 5.59 µg/mL; *V. amygdalina*: 67.15 ± 3.29 µg/mL) compared with the aqueous extracts. In anti-oocyst assays, hydroacetic extracts of *V. amygdalina* inhibited the sporulation of *E. magna* oocysts by up to 91% at 40 mg/mL ($p < 0.05$). Toxicity assays indicated no lethality in *A. salina* and no major histopathological alterations in rats. Collectively, these findings demonstrate that *O. gratissimum* and *V. amygdalina* extracts possess significant antioxidant and anticoccidial activities while exhibiting low toxicity, supporting their potential application in natural antioxidant and anticoccidial therapies. These results suggest that extracts of *O. gratissimum* and *V. amygdalina* have strong potential as natural antioxidants and anticoccidials with good biocompatibility, paving the way for their use in sustainable coccidiosis control strategies in livestock farming.

Keywords Natural coccidiostat, Oxidative stress, Medicinal plants, Bioactive substances, Biocompatibility

Abbreviations

%	Percentage
µg/ml	Micrograms per milliliter
AcidAsc	Ascorbic Acid
ALP	alkaline phosphatase

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ALT	Alanine aminotransaminase
AST	Aspartate Aminotransaminase
BP	Blood Patelets
CI	Confidence Interval
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
E.magna	<i>Eimeria magna</i>
E.media	<i>Eimeria media</i>
EAG	Gallic Acid Equivalent
EC	Catechin Equivalent
EC ₅₀	Half maximal effective concentration
ER	Rutin Equivalent
ES	Dry Extract
FCR	Folin-Ciocalteu reagent
fL	femtoliter
g/dL	Grams per deciliter
g/L	Grams per liter
h	Hour
HBSS	Hank's buffered salt solution
IC ₅₀	half-maximal inhibitory concentration
K ₂ Cr ₂ O ₇	Potassium dichromate
LaBAA	Laboratory of Biotechnology and Animal Improvement
LC ₅₀	Lethal concentration 50%
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
mg/dL	Milligrams per deciliter
mg/ml	Milligram per millilitre
mm ³	Cubic millimetres
Na ₂ CO ₃	Sodium carbonate
O. gratissimum	<i>Ocimum gratissimum</i>
OgAc	hydroacetic extract of <i>Ocimum gratissimum</i>
OgAq	Aqueous extract of <i>Ocimum gratissimum</i>
PCV	Packed Cell Volume
pg	Picograms
R ²	Rsquare
RBC	Red blood cells
U/L	Units per liter
V. amygdalina	<i>Vernonia amygdalina</i>
VaAc	hydroacetic extract of <i>Vernonia amygdalina</i>
VaAq	Aqueous extract of <i>Vernonia amygdalina</i>
WBC	White Blood Cells
γ-GT	Gamma-Glutamyl Transferase

Coccidial infections caused by *Eimeria* spp. are a major constraint in rabbit production, causing gastrointestinal disorders, impaired growth, and economic losses^{1,2}. *Eimeria magna* and *Eimeria media* are among the most prevalent species³. Although several anticoccidial drugs such as sulfonamides (e.g., sulfadimethoxine, sulfamethazine, sulfaquinoxaline), amprolium, toltrazuril, diclazuril, and ionophores (e.g., salinomycin, monensin) are commonly used to control coccidiosis in rabbits and poultry, their repeated use fosters the emergence of drug-resistant *Eimeria* strains⁴. Moreover, concerns about drug residues in meat and potential impacts on food safety have been raised, particularly in intensive production systems^{5,6}. These limitations highlight the urgent need for safer, sustainable alternatives such as plant-derived anticoccidials.”

Ocimum gratissimum and *Vernonia amygdalina* are widely used in traditional medicine in Africa and Asia and are rich in bioactive compounds such as flavonoids, tannins, alkaloids, saponins, and essential oils^{7,8}, conferring antioxidant, antimicrobial, and antiparasitic properties.

Ocimum gratissimum is rich in phenolic compounds, notably eugenol, which exhibits antioxidant, anti-inflammatory, and antimicrobial properties⁹. Its essential oils confer strong antioxidant and antimicrobial activities^{10,11}, suggesting potential efficacy against protozoan pathogens, though its anticoccidial effects remain largely unexplored. Despite a relatively abundant literature on the general antimicrobial and antiparasitic properties of *O. gratissimum*, data on its anticoccidial activity against *Eimeria* spp. remain limited and inconclusive. To date, no in vivo reference studies have robustly confirmed its anticoccidial efficacy or precisely elucidated its potential mechanisms of action. This situation highlights a scientific gap that warrants exploration through more systematic and rigorous experimental work. *Vernonia amygdalina* (“bitter leaf”) contains saponins, alkaloids, and flavonoids with antiparasitic, immunomodulatory, and antioxidant activities^{8,12}. Saponins can disrupt parasite membranes or modulate host immunity¹³, and the leaf are traditionally used to treat gastrointestinal infections¹⁴. Together, *O. gratissimum* and *V. amygdalina* offer complementary antimicrobial, antioxidant, and antiparasitic activities, potentially acting synergistically against *E. magna* and *E. media*. However, their efficacy, mechanisms of action, and safety in rabbits remain insufficiently studied. Because plant extracts can include toxic compounds, it is necessary to evaluate both cytotoxicity and acute toxicity^{9,15}. Such evaluations help define

safe dosage ranges and prevent adverse effects on host tissues, metabolism, and organ function. Despite these promising attributes, critical gaps remain regarding their anticoccidial activity against *E. magna* and *E. media*. Specifically, the mechanisms of action are poorly understood: it is unclear whether inhibition of sporulation and sporozoite viability results from direct disruption of parasite membranes or indirect modulation of host immunity. Moreover, while preliminary toxicity assays in rodents suggest good tolerance, the safety profile in rabbits remains largely undocumented, particularly concerning chronic exposure, organ-specific effects, and impacts on growth and reproduction. Previous studies in rabbit feeding trials have reported zootechnical and hematological benefits of these plants^{16,17}, but they do not provide mechanistic insights or robust toxicological evaluations. This situation highlights the need for systematic investigations to clarify efficacy, mechanisms, and safety in rabbits.

This study evaluates the antioxidant, in vitro anticoccidial, and toxicity profiles of *O. gratissimum* and *V. amygdalina* leaf extracts against *E. magna* and *E. media*. Phytochemical characterization was performed to identify bioactive compounds linked to these effects. The key questions are: (1) Do these extracts inhibit *Eimeria* spp. in vitro, and by which mechanisms? (2) What are their larval cytotoxicity and acute toxicity profiles? We hypothesize that the extracts exhibit significant antioxidant and anticoccidial activities, with minimal toxicity, attributable to bioactive compounds such as flavonoids, alkaloids, and tannins.

This study aims to develop safer, sustainable alternatives to conventional anticoccidial drugs. Specifically, it (i) evaluates the in vitro antioxidant and anticoccidial activity of *O. gratissimum* and *V. amygdalina* against *E. magna* and *E. media*, (ii) characterizes their bioactive compounds, and (iii) assesses larval cytotoxicity and acute oral toxicity. The goal is to provide a scientific basis for using these plants as natural anticoccidial agents, supporting sustainable rabbit production and reducing drug resistance.

Results
Phytochemical screening

Qualitative phytochemical screening revealed that the leaf of *O. gratissimum* and *V. amygdalina* contain several common chemical groups, suggesting overlapping biological properties. Both species contained tannins (catechic and gallic), flavonoids, quinone derivatives, coumarins, reducing compounds, O-heterosides (reduced and unreduced), C-heterosides, and alkaloids. Notable differences were observed: leucoanthocyanins were absent in *O. gratissimum* but present in *V. amygdalina*, while saponosides were absent in *O. gratissimum* but occurred at relatively high levels in *V. amygdalina* (foam height > 1 cm). Free anthracenics and cyanogenic derivatives were absent in both plants (Table 1).

Polyphenol and flavonoid contents of *O. gratissimum* and *V. amygdalina* powder leaf

Quantitative analysis revealed that the hydroacetic extract of *V. amygdalina* leaf (VaAc) contained the highest total phenolic content (736.27 ± 0.97 mg GAE/g extract), followed by the hydroacetic extract of *O. gratissimum* leaf (OgAc; 549.85 ± 1.55 mg GAE/g extract; *p* < 0.05). The aqueous extracts of *V. amygdalina* (VaAq) and *O. gratissimum* (OgAq) exhibited lower total phenolic contents, 420.35 ± 1.94 mg GAE/g and 374.48 ± 0.58 mg GAE/g extract, respectively (*p* < 0.05).

Flavonoid content was highest in the hydroacetic extract of *O. gratissimum* (37.06 ± 0.31 mg RE/g extract), followed by the hydroacetic extract of *V. amygdalina* (30.71 ± 0.68 mg RE/g extract). The aqueous extracts of *O. gratissimum* (18.89 ± 0.48 mg RE/g extract) and *V. amygdalina* (16.74 ± 0.19 mg RE/g extract) had the lowest flavonoid levels (*p* < 0.05).

	<i>O. gratissimum</i>	<i>V. amygdalina</i>
Tannins	+	+
Catechin tannins	+	+
Gallic tannins	+	+
Leucoanthocyanins	-	+
Flavonoids	+	+
Quinone derivatives	+	+
Saponosides	-	+ > 1 cm
Mucilages	+	+
Coumarins	+	+
Reducing compounds	+	+
Free anthracenics	-	-
O-heterosides	+	+
Reduced genine O-heterosides	+	+
C-heterosides	+	+
Alkaloids	+	+
Cyanogenic derivatives	-	-

Table 1. Qualitative screening of *O. gratissimum* and *V. amygdalina* powder leaf. “+” = presence; “-” = absence; >1 cm represents the height of the lather created.

Extrait	<i>O. gratissimum</i>		<i>V. amygdalina</i>		Equations	R ²	Pvalue
	OgAq	OgAc	VaAq	VaAc			
Total phenols (mg EAG/g ES)	374.48 ± 0.58a	549.85 ± 1.55d	420.35 ± 1.94c	736.27 ± 0.97f	y = 0.115x + 0.003	0.998	< 0.001
Flavonoids (mg ER/g ES)	18.89 ± 0.48b	37.06 ± 0.31e	16.74 ± 0.19a	30.71 ± 0.68c	y = 0.160x + 0.006	0.9993	< 0.001
Condensed tannins (mg EC/g ES)	1.53 ± 0.12a	2.83 ± 0.13a	3.53 ± 0.52b	5.94 ± 0.42d	y = 0.129x + 0.005	0.9953	< 0.001

Table 2. Contents of total phenols, flavonoids and condensed tannins in *O. gratissimum* and *V. amygdalina* leaf extracts. The values bearing the different letters a, b, c, d, and e in the same line are significantly different at the 5% level ($p < 0.05$) for the same metabolite. OgAq: Aqueous extracts of *O. gratissimum*, OgAc: hydroacetic extract of *O. gratissimum*, VaAq: Aqueous extract of *V. amygdalina*, VaAc: hydroacetic extract of *V. amygdalina*, EAG: gallic acid equivalent, ER: rutin equivalent, EC: catechin equivalent, ES: dry extract; x: Optical density. Results are presented as mean ± standard deviation (Mean ± SD).

	IC ₅₀	CI	R square
Acid Asc	24.92 ± 1.93	19.953 to 29.89	0.988
VaAc	67.15 ± 3.29	59.11 to 75.19	0.998
OgAc	51.2 ± 5.59	36.82 to 65.57	0.991
VaAq	117.45 ± 9.28	93.59 to 141.31	0.995
OgAq	99.55 ± 11.92	68.92 to 130.18	0.99

Table 3. Free radical scavenging activity of mature leaf of *O. gratissimum* and *V. amygdalina* using the DPPH assay. OgAq: Aqueous extracts of *O. gratissimum*, OgAc: hydroacetic extract of *O. gratissimum*, VaAq: Aqueous extract of *V. amygdalina*, VaAc: hydroacetic extract of *V. amygdalina*, Acid Asc: Ascorbic acid, EC₅₀: Effective Concentration 50%; CI: Confidence Interval at 95%; DPPH: 2,2-diphenyl-1-picrylhydrazyl. Results are presented as mean ± standard deviation (Mean ± SD).

Condensed tannins were most abundant in the hydroacetic extract of *V. amygdalina* (5.94 ± 0.42 mg CE/g extract), followed by its aqueous extract (3.53 ± 0.52 mg CE/g extract). Both aqueous and hydroacetic extracts of *O. gratissimum* contained lower tannin levels, 2.83 ± 0.13 mg CE/g and 1.53 ± 0.12 mg CE/g extract, respectively ($p < 0.05$; Table 2).

Antioxidant activity

The EC₅₀ values obtained from the DPPH radical scavenging assay indicate marked variability in antioxidant activity among the extracts studied. Ascorbic acid, used as a positive control, has the lowest EC₅₀ (24.92 ± 1.93 µg/mL), confirming its strong antioxidant capacity. Among the plant extracts, the hydroacetic extracts of *V. amygdalina* (67.15 ± 3.29 µg/mL) and *O. gratissimum* (51.20 ± 5.59 µg/mL) showed significantly higher activity than their respective aqueous extracts, which had higher EC₅₀ values. This difference highlights the increased efficiency of the hydro-organic solvent for the extraction of phenolic compounds and flavonoids, which are generally responsible for antioxidant activity. Furthermore, the relatively narrow confidence intervals and high coefficients of determination ($R^2 \geq 0.98$) attest to the robustness of the dose-response models and the reliability of the estimated EC₅₀ values (Table 3).

In vitro oocysticidal activities of *O. gratissimum* and *V. amygdalina* leaf extracts

At low concentrations (2.5 mg/mL), all extracts exhibited limited sporulation inhibition. The hydroacetic extract of *V. amygdalina* leaf (VaAc) demonstrated the highest activity, with inhibition percentages of 35.33% for *E. magna* at 24 h and 44.67% for *E. media* at 48 h, while other extracts showed significantly lower effects ($p < 0.05$). At intermediate concentrations (5–10 mg/mL), inhibition increased proportionally with extract concentration. Hydroacetic extracts of both *V. amygdalina* and *O. gratissimum* leaf remained significantly more effective ($p < 0.05$), reaching 62.67% (*E. magna*, 24 h) for VaAc at 5 mg/mL and up to 82.33% (*E. magna*, 24 h) for the hydroacetic extract of *O. gratissimum* leaf (OgAc) at 10 mg/mL.

At high concentrations (20–40 mg/mL), inhibition approached maximal levels, particularly for VaAc at 40 mg/mL, achieving 91% inhibition at 24 h and 100% at 48 h against *E. magna*. Comparable results were observed for *E. media* (Table 4). In contrast, aqueous extracts were generally less effective than hydroacetic extracts. The positive control (5% phenol) produced nearly complete inhibition (97.67–100%) for both species, confirming the upper efficacy limit. The negative control (K₂Cr₂O₇ alone or with DMSO) showed minimal inhibition (6–9%), indicating that the observed effects were primarily attributable to bioactive plant compounds.

Overall, hydroacetic extracts, particularly from *V. amygdalina* leaf, were the most effective against both *Eimeria* species across all tested concentrations, with high-concentration treatments approaching the efficacy of the positive control. Aqueous extracts exhibited moderate activity, with VaAq slightly more effective than OgAq. Inhibition of oocyst sporulation increased with extract concentration, and at 20–40 mg/mL, the effect was comparable to or equal to that of the positive control (Table 4).

Concentration (mg/ml)	Extracts	Incubation times for Eimeria strains			
		E. magna		E. media	
		24 hours	48 hours	24 hours	48 hours
2.5	OgAq	13.67±4.73 ^a	28.33±3.21 ^a	12.00±3.00 ^a	25.67±8.96 ^a
	VaAq	18.00±5.29 ^{ab}	36.33±4.16 ^{abc}	14.33±2.89 ^a	37.33±6.43 ^{ab}
	OgAc	23.67±1.53 ^b	30.00±4.00 ^a	19.33±1.53 ^b	34.67±9.24 ^{ab}
	VaAc	35.33±4.51 ^c	43.33±3.79 ^c	22.33±0.58 ^b	44.67±4.51 ^b
5	OgAq	33.33±4.16 ^a	58.00±2.65 ^a	33.00±4.00 ^a	54.00±9.54 ^a
	VaAq	46.00±5.29 ^b	66.00±5.57 ^{ab}	40.00±3.61 ^a	67.33±7.02 ^{ab}
	OgAc	49.67±3.51 ^b	60.33±4.73 ^a	39.33±2.89 ^a	63.33±11.55 ^{ab}
	VaAc	62.67±10.21 ^c	71.00±2.65 ^b	51.00±1.73 ^b	73.33±3.79 ^b
10	OgAq	51.00±1.00 ^a	78.33±10.21 ^{ab}	49.00±6.00 ^a	75.33±11.85 ^a
	VaAq	64.33±6.81 ^b	85.33±4.04 ^{ab}	64.00±1.00 ^{bc}	86.33±5.69 ^{ab}
	OgAc	69.00±6.93 ^b	81.33±4.73 ^{ab}	60.00±2.65 ^b	85.00±8.72 ^{ab}
	VaAc	82.33±10.02 ^c	90.00±2.00 ^b	70.33±0.58 ^{bc}	91.67±5.13 ^b
20	OgAq	55.00±5.00 ^a	83.00±8.89 ^a	54.00±7.00 ^a	80.67±9.61 ^a
	VaAq	69.00±4.58 ^b	90.33±3.21 ^{ab}	68.33±1.53 ^{bc}	92.33±1.53 ^b
	OgAc	73.67±7.77 ^b	85.33±6.66 ^{ab}	63.67±3.51 ^{ab}	87.33±9.29 ^{ab}
	VaAc	88.00±7.21 ^c	94.67±1.53 ^c	78.00±6.08 ^c	96.67±4.16 ^b
40	OgAq	62.00±6.56 ^a	87.00±8.72 ^a	57.33±6.51 ^a	84.00±8.89 ^a
	VaAq	71.67±3.79 ^{ab}	93.00±4.58 ^{ab}	73.00±1.00 ^b	95.00±2.65 ^b
	OgAc	78.00±8.19 ^b	91.00±6.24 ^b	68.33±3.51 ^b	90.67±7.77 ^{ab}
	VaAc	91.00±2.65 ^c	100.00±0.00 ^b	84.33±5.13 ^c	100.00±0.00 ^b
Negative control	K ₂ Cr ₂ O ₇ +DMSO	6±1.73	6.33±1.53	7±2.65	5.66±152
	K ₂ Cr ₂ O ₇	9±2	9.67±0.58	9±1	9.00±2.00
Positivve control	5%	97.67±2.52	100.00±0.00	97.00±3.00	100.00±0.00

Table 4. Effect of *O. gratissimum* and *V. amygdalina* on the Inhibition of Oocyst Sporulation of *E. imeria*. *magna* and *E.imeria media*. The values bearing the different letters a, b, c, d, and e in the same column are significantly different at the 5% level ($p < 0.05$) for the same metabolite. OgAq: Aqueous extracts of *O. gratissimum*, OgAc: hydroacetic extract of *O. gratissimum*, VaAq: Aqueous extract of *V. amygdalina*, VaAc: hydroacetic extract of *V. amygdalin*. Results are presented as mean ± standard deviation (Mean ± SD)

	Df	Sum Sq	Mean Sq	F value	Pr(> F)	value
Dose	4	100,983	25,246	704.450	< 0.001	***
Time	1	21,603	21,603	602.805	< 0.001	***
Extracts	3	11,813	3938	109.872	< 0.001	***
Dose: Time	4	220	55	1.534	0.194	
Dose: Extracts	12	154	13	0.358	0.976	
Time: Extracts	3	905	302	8.420	< 0.001	***
Dose: Time: Extracts	12	296	25	0.689	0.761	
Residuals	200	7167	36			

Table 5. Three-way ANOVA of the effects of dose, time, and plant extracts on Eimeria oocyst sporulation inhibition. Signif.codes: ‘***’0.001; ‘**’0.01; ‘*’0.05; Sq : Square.

This table (Table 5) presents the results of a three-factor ANOVA designed to evaluate the impact of dose, exposure time, and type of plant extract on the inhibition of oocyst sporulation. The main effects are all highly significant: dose ($F=704.450$), time ($F=602.805$), and extracts ($F=109.872$) strongly influence the biological response ($p < 0.001$). The interaction between time and extracts is also significant ($F=8.420$, $p < 0.001$), suggesting that the effectiveness of the extracts varies depending on the duration of exposure. However, the dose × time, dose × extracts, and triple dose × time × extracts interactions are not significant ($p > 0.05$), indicating that these combinations have no notable synergistic effect on oocyst viability. The low level of residuals (Mean Sq = 36) confirms the robustness of the model.

For all extracts (Fig. 1), the average inhibition of oocyst sporulation increases with dose (2.5 → 40 µg/mL). This shows a clear dose-dependent effect. At 48 h, inhibition is generally higher for each dose and extract, confirming the effect of time on sporulation inhibition. Va_Ac shows the highest inhibition at all doses and at

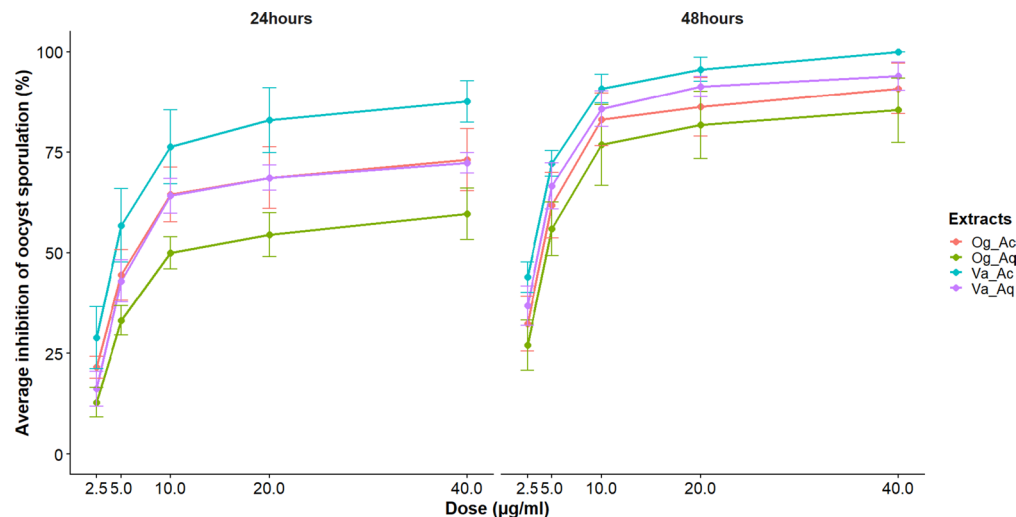


Fig. 1. Dose-response curves for the inhibition of sporulation oocysts by extracts of *O. gratissimum* and *V. amygdalina* after 24 and 48 h. OgAq: Aqueous extract of *O. gratissimum*, OgAc: hydroacetic extract of *O. gratissimum*, VaAq: Aqueous extract of *V. amygdalina*, VaAc: hydroacetic extract of *V. amygdalina*.

each time point, followed by Va_Aq and Og_Ac. The weakest effect, particularly at low doses, is obtained by Og_Aq (Fig. 1).

In vitro anti-sporozooidal activities of *O. gratissimum* and *V. amygdalina* leaf extracts

The inhibitory efficacy of all extracts increased significantly with concentration ($p < 0.05$), irrespective of extract type or *Eimeria* species. At 125 µg/mL, viability inhibition of *E. magna* and *E. media* was relatively low, with the hydroacetic extract of *V. amygdalina* (VaAc) showing the greatest effect ($29.00 \pm 2.65\%$ for *E. magna* at 12 h; $47.33 \pm 6.03\%$ at 24 h). At 250 µg/mL, inhibition increased for all extracts, with VaAc maintaining superior activity ($47.00 \pm 2.00\%$ at 12 h; $65.67 \pm 1.15\%$ at 24 h against *E. magna*). At 500 µg/mL, the efficacy of the aqueous extracts approached that of hydroacetic extracts, though VaAc remained the most potent ($91.00 \pm 1.73\%$ against *E. media* at 24 h). At 1000 µg/mL, inhibition reached near-complete or complete levels, particularly for hydroacetic extracts of *V. amygdalina* and *O. gratissimum*, confirming a clear dose-dependent response. *Eimeria magna* appeared slightly more sensitive to the extracts, especially VaAc at 24 h, achieving 100% inhibition at 1000 µg/mL, equivalent to the positive control. *Eimeria media* followed a similar trend, though inhibition values were marginally lower across most concentrations (Table 6).

This table (Table 7) presents the results of a three-factor ANOVA evaluating the impact of dose, time, and extract type on the inhibition of coccidian oocyst viability. The main effects of dose ($F = 1164.205$), time ($F = 322.488$), and extracts ($F = 190.147$) are all highly significant ($p < 0.001$), indicating that each of these factors strongly influences the measured response. The interaction between dose and time is not significant ($p = 0.268$), suggesting that the effect of dose is constant regardless of the time of observation. In contrast, the dose $\times$ extract interaction is significant ($F = 5.419$, $p < 0.001$), revealing that the effect of dose varies depending on the type of extract used. The time $\times$ extract interaction is marginally insignificant ($p = 0.083$), while the triple dose $\times$ time $\times$ extract interaction is significant ($F = 2.645$, $p = 0.007$), indicating complexity in the combined response of the three factors. The low value of the residuals (Mean Sq = 31) indicates a good fit of the model studied (Table 7).

This figure (Fig. 2) illustrates the increasing effect of different doses (125 to 1000 µg/mL) of four plant extracts on the viability of coccidian oocysts. There is a general trend toward increased inhibition of oocyst viability with increasing dose, indicating a dose-dependent relationship. Acetic extracts (Og_Ac and Va_Ac) appear to be more effective than aqueous extracts, achieving inhibition levels close to 100% at the maximum dose. This efficacy varies slightly between the two species, suggesting differential sensitivity. These results highlight the antiparasitic potential of plant extracts, particularly those obtained by acetic extraction, in the control of coccidia (Fig. 2).

Dose-response curves revealed a clear inverse relationship between extract concentration and oocyst sporulation, characteristic of antiparasitic agents. IC_{50} values, representing the concentrations required to inhibit 50% of oocyst sporulation, were determined via regression analysis (Fig. 3). For *O. gratissimum* leaf extracts, the aqueous and hydroacetic preparations inhibited 50% of *E. media* oocysts at 3.466 mg/mL ($R^2 = 0.9749$) and 4.413 mg/mL ($R^2 = 0.9873$), respectively (Fig. 3A, B). In comparison, *V. amygdalina* leaf extracts achieved 50% inhibition at lower concentrations: 2.920 mg/mL for the aqueous extract and 3.415 mg/mL for the hydroacetic extract, with R^2 values of 0.9873 and 0.9817, respectively (Fig. 3C, D). The logarithmic regression model provided the highest coefficients of determination, supporting the reliability of the IC_{50} estimates. These results indicate that *V. amygdalina* extracts exert stronger anticoccidial activity than *O. gratissimum*, with the hydroacetic extract demonstrating slightly greater efficacy than the aqueous extract.

Figure 4 illustrates the inhibitory effects of leaf extracts on the sporulation of *E. magna* oocysts. IC_{50} values and R^2 coefficients were used to evaluate the potency and goodness-of-fit of the dose-response curves. High R^2 values (> 0.97) across all graphs indicate a strong correlation between extract concentration and reduction in

Concentration µg/ml	Extracts	Incubation times for Eimeria strains			
		E. magna		E. media	
		12 hours	24 hours	12 hours	24 hours
125	OgAq	5.67±1.15 ^c	14.67±3.79 ^a	6.33±1.53 ^c	13.33±3.21 ^a
	VaAq	11.67±2.52 ^{bc}	23.67±6.51 ^a	13.33±2.08 ^b	24.33±8.14 ^{ab}
	OgAc	16.67±5.69 ^{ba}	33.33±7.02 ^b	16.67±2.52 ^b	32.00±10.39 ^{bc}
	VaAc	29.00±2.65 ^a	47.33±6.03 ^c	31.00±4.58 ^d	46.00±1.73 ^d
250	OgAq	23.33±7.02 ^a	39.00±2.65 ^c	23.00±3.61 ^a	38.67±8.62 ^a
	VaAq	36.00±5.57 ^b	51.33±11.06 ^{cd}	32.67±3.21 ^{ab}	51.33±6.43 ^b
	OgAc	35.67±4.04 ^b	44.33±0.58 ^{de}	35.00±6.56 ^{abc}	43.67±1.15 ^{ab}
	VaAc	47.00±2.00 ^c	65.67±1.15 ^a	46.33±4.93 ^c	67.00±3.00 ^c
500	OgAq	50.67±4.62 ^d	58.67±4.73 ^a	49.67±6.66 ^b	57.67±7.37 ^c
	VaAq	63.00±6.24 ^{bc}	88.33±11.72 ^c	63.67±8.50 ^a	85.00±5.29 ^{ab}
	OgAc	52.33±8.33 ^{cd}	72.67±11.50 ^b	48.33±9.45 ^b	73.00±8.19 ^b
	VaAc	77.00±5.20 ^a	89.67±0.58 ^c	78.00±10.54 ^a	91.00±1.73 ^a
1000	OgAq	65.00±4.58 ^a	77.67±2.52 ^a	66.67±9.07 ^c	78.67±6.66 ^c
	VaAq	80.67±7.64 ^{bc}	93.67±1.53 ^c	83.67±9.50 ^{ab}	97.00±3.00 ^{ab}
	OgAc	74.67±7.57 ^{ab}	88.67±4.16 ^b	73.00±8.89 ^{bc}	92.67±5.51 ^b
	VaAc	91.67±2.08 ^c	100.00±0.00 ^d	90.67±11.15 ^a	100.00±0.00 ^a
Negative control	DMSO	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	HBSS	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Positive control	50 µg/ml	88.67±3.51	100.00±0.00	89.00±1.73	100.00±0.00

Table 6. Percent inhibition of viability of *E. magna* and *E. media* strains in contact with *O. gratissimum* and *V. amygdalin* extracts. OgAq: Aqueous extract of *O. gratissimum*, OgAc: hydroacetonic extract of *O. gratissimum*, VaAq: Aqueous extract of *V. amygdalina*, VaAc: hydroacetonic extract of *V. amygdalina*, DMSO: Dimethyl sulfoxide, HBSS: Hank’s buffered salt solution. The results are presented as the means and standard deviations of triplicate *in vitro* tests after 12 h and 24 h of incubation at room temperature. Results are presented as mean ± standard deviation (Mean ± SD). For the same column and concentration, values with the same superscript letter are not significantly different at $p \geq 0.05$ (Duncan’s test)

	Df	Sum Sq	Mean Sq	F value	Pr(> F)	value
Dose	3	108,358	36,119	1164.205	< 0.001	***
Time	1	10,005	10,005	322.488	< 0.001	***
Extract	3	17,698	5899	190.147	< 0.001	***
Dose: Time	3	123	41	1.326	0.268	
Dose: Extract	9	1513	168	5.419	< 0.001	***
Time: Extract	3	211	70	2.262	0.083	
Dose: Time: Extract	9	739	82	2.645	0.007	**
Residuals	160	4964	31			

Table 7. Three-way ANOVA on the effect of doses, time, and extracts of *O. gratissimum* and *V. amygdalina* on the inhibition of oocyst viability. Signif. codes: ‘***’, 0.001; ‘**’, 0.01; ‘*’, 0.05; Sq : Square.

oocyst sporulation. Extracts of *V. amygdalina* (Fig. 4C, D) exhibited greater efficacy than those of *O. gratissimum* (Fig. 4A, B), with lower IC₅₀ values of 3.097 mg/mL (hydroacetone) and 3.408 mg/mL (aqueous), reflecting superior anticoccidial activity independent of extraction method. In contrast, *O. gratissimum* extracts displayed slightly higher IC₅₀ values 4.066 mg/mL (hydroacetone) and 4.091 mg/mL (aqueous) indicating moderate efficacy. These findings highlight *V. amygdalina* as the more potent inhibitor of *E. magna* oocyst sporulation (Fig. 4).

Larval toxicity of extracts

Larval toxicity assays indicated that all tested plant extracts were non-toxic to *A. salina* larvae, with some variation between aqueous and hydroacetonic extracts (Fig. 5). The aqueous extract of *V. amygdalina* (Fig. 5A) exhibited an LC₅₀ of 0.269 mg/mL ($R^2 = 0.93$), classifying it as nontoxic. Similarly, the aqueous extract of *O. gratissimum* (Fig. 5C) had an LC₅₀ of 0.168 mg/mL ($R^2 = 0.95$), also nontoxic, reflecting a robust model fit. Hydroacetonic extracts showed comparable profiles: *V. amygdalina* had an LC₅₀ of 0.978 mg/mL ($R^2 = 0.92$; Fig. 5B), while *O. gratissimum* displayed an LC₅₀ of 0.137 mg/mL ($R^2 = 0.76$; Fig. 5D). Although the latter’s

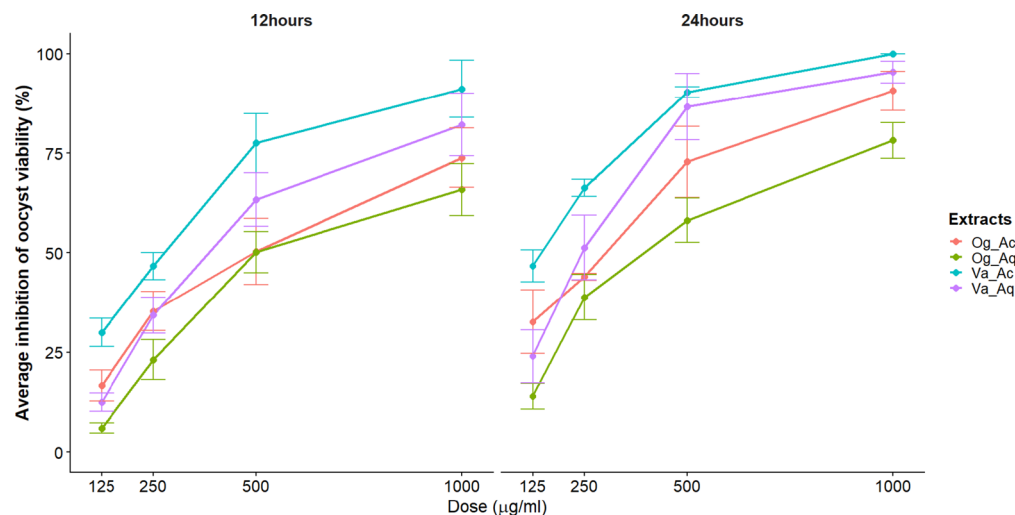


Fig. 2. Dose-response curves for the inhibition of the viability of *E. magna* and *E. media* oocysts by extracts of *O. gratissimum* and *V. amygdalina* after 12 h and after 24 h. OgAq: Aqueous extract of *O. gratissimum*, OgAc: hydroacetic extract of *O. gratissimum*, VaAq: Aqueous extract of *V. amygdalina*, VaAc: hydroacetic extract of *V. amygdalina*.

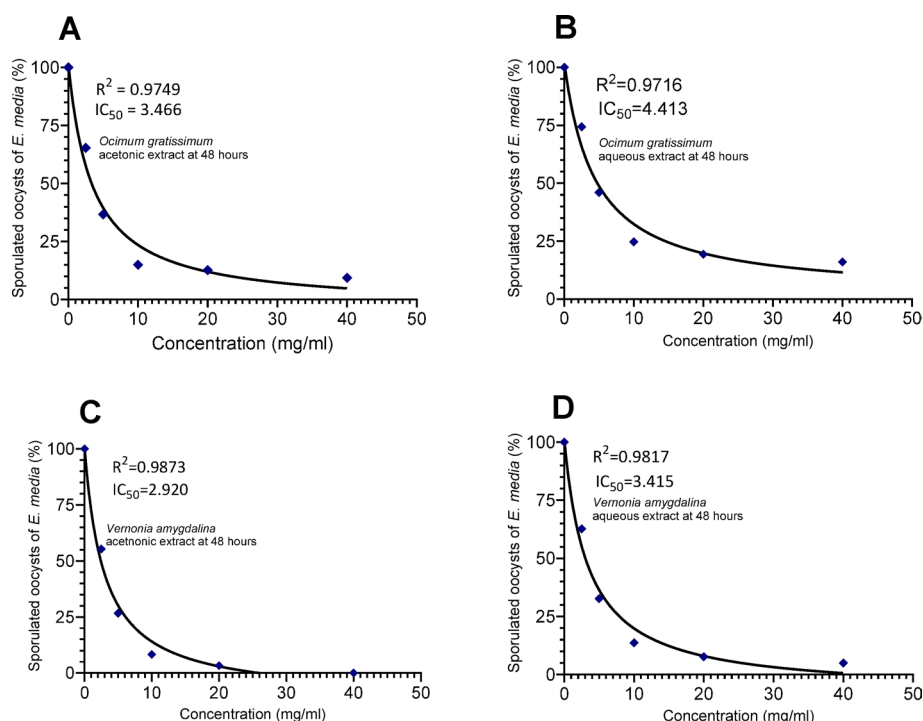


Fig. 3. Logarithmic correlations between the percentage of sporulated oocysts in *E. media* and the concentrations of *O. gratissimum* leaf hydroacetic extract (A), *O. gratissimum* leaf aqueous extract (B), *V. amygdalina* leaf hydroacetic extract (C), and *V. amygdalina* leaf aqueous extract (D).

lower R^2 suggests some variability in the regression fit, it remains within the nontoxic range. Overall, both plants produced extracts with negligible larval toxicity, with LC_{50} values influenced by the extraction solvent (Fig. 5).

Acute oral toxicity

Mortality and clinical signs

No mortality was observed in the rats treated with the hydroacetic extracts of the three plants during the acute toxicity tests. No signs or abnormalities were recorded on the skin, coat, eyes or mucous membranes. There was also a complete absence of hyperactivity, eye twitches, catalepsy, convulsions, tremors and catatonia following

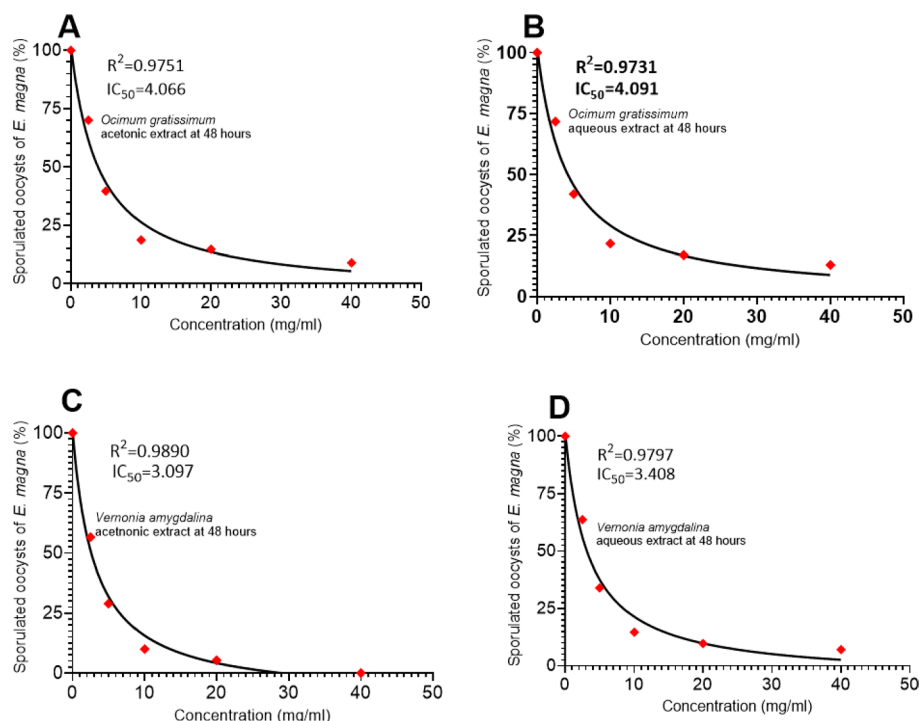


Fig. 4. The logarithmic correlation between the percentage of sporulated oocysts of *E. magna* and the concentrations of *O. gratissimum* leaf hydroacetonic extract (A), *O. gratissimum* leaf aqueous extract (B), *V. amygdalina* leaf hydroacetonic extract (C), and *V. amygdalina* leaf aqueous extract (D).

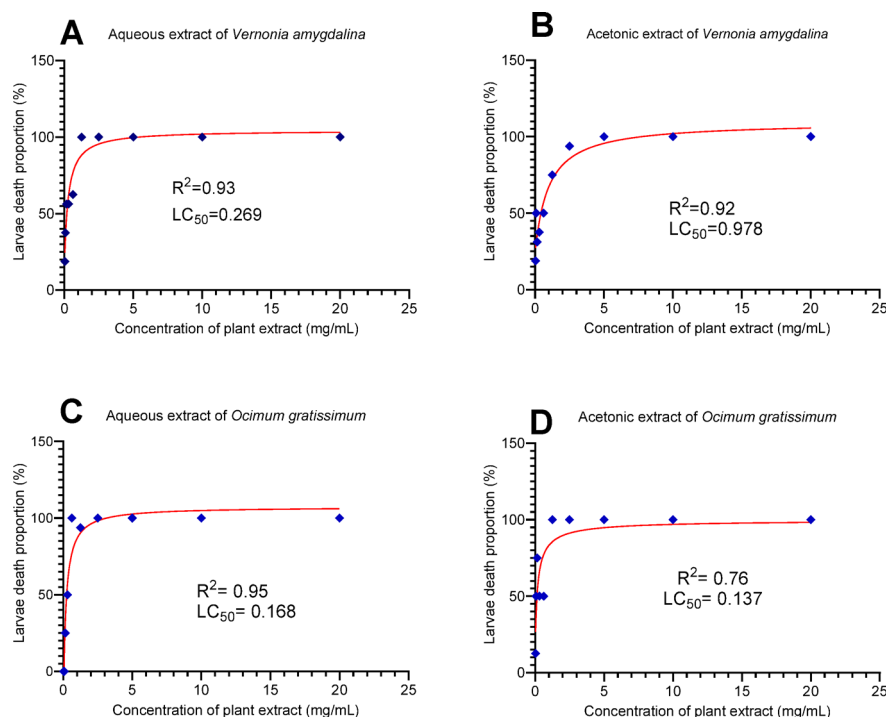


Fig. 5. The logarithmic correlation between the percentage of death of *A. salina* larvae and the concentration of *V. amygdalina* leaf aqueous extract (A), *V. amygdalina* leaf hydroacetonic extract (B), *O. gratissimum* leaf aqueous extract (C), and *O. gratissimum* leaf hydroacetonic extract (D).

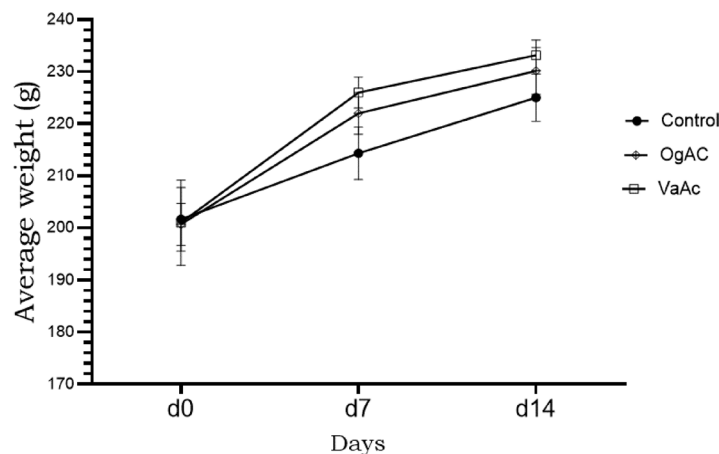


Fig. 6. Effect of *O. gratissimum* and *V. amygdalina* extracts on weight growth of treated rats.

	Control	OgAc	VaAc	P value
WBC ($10^3/\mu\text{L}$)	13.98 ± 0.38	12.7 ± 0.9	13.25 ± 0.54	0.08
RBC ($10^6/\mu\text{L}$)	7.35 ± 0.55	7.76 ± 0.02	6.63 ± 0.1	0.061
Hb (g/dL)	14.43 ± 0.43	14.5 ± 0	13.35 ± 0.05	0.055
PCV (%)	43.35 ± 0.35	48.4 ± 0.2	47.2 ± 1.8	0.061
MCV (fL)	62.55 ± 1.05	62.4 ± 0.1	71.15 ± 1.65	0.061
MCH (pg)	23 ± 1^a	18.7 ± 0^b	20.15 ± 0.35^{ab}	0.027
MCHC (g/dL)	34.85 ± 1.15^a	29.95 ± 0.15^b	28.3 ± 1.2^b	0.024
BP ($10^3/\mu\text{L}$)	745.5 ± 5.5^a	578 ± 13^b	793.5 ± 3.5^a	0.027

Table 8. Hematological Effects of *O. gratissimum* and *V. amygdalina* extracts in treated rats. RBC = Red blood cells. PCV = Packed cell volume. MCV = mean corpuscular volume. MCH = mean corpuscular haemoglobin. MCHC = mean corpuscular haemoglobin concentration. WBC = white blood cell. OgAc: hydroacetic extract of *O. gratissimum*. VaAc: hydroacetic extract of *V. amygdalina*. Results are presented as mean $\pm$ standard deviation (Mean $\pm$ SD).

treatment with the hydroacetic fractions of the plants. Administration of the hydroacetic extracts produced no signs of erythema or edema until after 48 h of observation.

Food consumption and body weight of the rats

There were no significant differences in food consumption between the test group and the control group. The weights of the subjects in the treated batches differed significantly ($p < 0.05$) from those in the control batches at D7 and D14 (Fig. 6).

Hematological parameters of the rats

Analysis of hematological parameters revealed significant alterations among the three experimental groups (Table 8). Most values including WBC, RBC, Hb, and PCV show slight variations among groups, with p-values hovering just above the significance threshold (0.055–0.08), suggesting potential trends without statistical confirmation. However, significant differences are observed in red blood cell indices: MCV is markedly elevated in the VaAc group ($p < 0.001$), indicating larger cell volume, while MCH and MCHC are significantly reduced in treated groups ($p = 0.027$ and 0.024), pointing to altered hemoglobin content and concentration. Platelet counts (BP) also differ significantly ($p = 0.027$), with OgAc showing a notable decrease and VaAc an increase compared to control (Table 8).

Biochemical parameters of the rats

Glucose levels show a slight reduction in treated groups, but the difference is not statistically significant ($p = 0.099$). Urea levels are elevated in the OgAc group, with a borderline p-value (0.067). Creatinine levels are slightly higher in treated groups, especially VaAc, though not significantly ($p = 0.329$). Notably, ALT activity is significantly increased in both extract-treated groups ($p = 0.027$), suggesting possible hepatic stress or enzyme induction. AST levels are highest in the VaAc group, with a p-value close to significance (0.061), while ALP and γ -GT activities show no significant changes across treatments ($p = 0.201$ and 0.148 , respectively), (Table 9).

	Control	OgAc	VaAc	pvalue
Glucose (g/L)	0.72 ± 0.05	0.67 ± 0.03	0.66 ± 0.01	0.099
Urea (g/L)	0.20 ± 0.01	0.33 ± 0.08	0.24 ± 0.04	0.067
Creatinine (mg/L)	10.12 ± 1.85	11.51 ± 0.48	12.46 ± 2.48	0.329
ALT (U/L)	66.58 ± 2.02 ^b	92.08 ± 1.01 ^a	98.66 ± 0.74 ^a	0.027
AST (U/L)	124.33 ± 5.09	121.13 ± 2.13	148.38 ± 0.96	0.061
ALP (U/L)	446.83 ± 27.05	477.43 ± 31.75	419.82 ± 28.20	0.201
γ-GT (U/L)	3.85 ± 0.08	3.47 ± 0.42	4.52 ± 0.67	0.148

Table 9. Biochemical parameters of the rats. Biochemical impact of *O. gratissimum* and *V. amygdalina* extracts on liver and renal function markers in treated rats. AST=aspartate amino transaminase. ALT=alanine amino transaminase. ALP=alkaline phosphate, γ-GT= Gamma-Glutamyl Transferase. Results are presented as mean ± standard deviation (Mean ± SD).

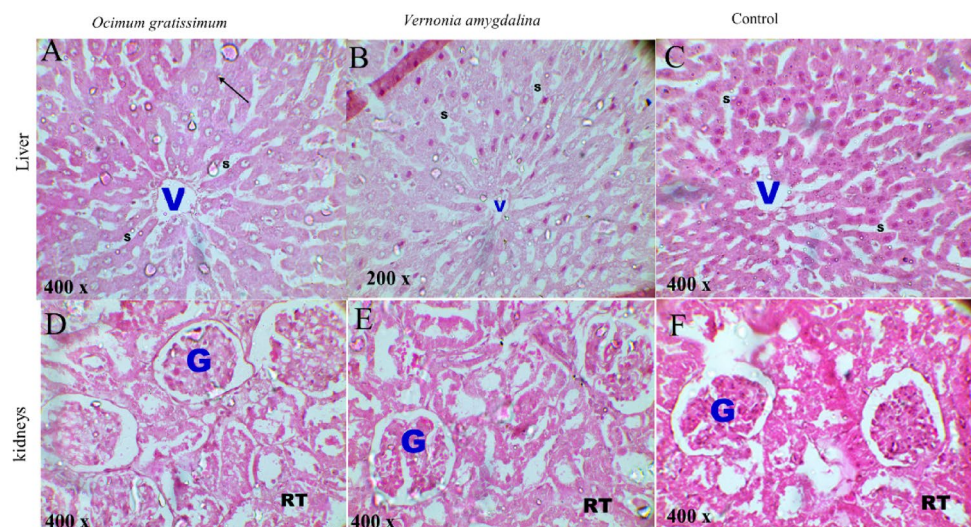


Fig. 7. Histological sections of the internal organs of rats fed different hydroacetic extracts of the two plants by single gavage observation at 400x magnification: (A) liver of rats fed with *O. gratissimum* extract; (B) liver of rats fed with *V. amygdalina* extract; (C) liver of control rats; (D) kidney of rats fed with *O. gratissimum* extract; (E) kidney of rats fed with *V. amygdalina* extract; (F) kidney of control rats. (V) Centrilobular vein; (G) glomerulus; (RT) renal tubules; (S) Sinusoids; $\diamond$ Nucleus of a hepatocyte.

Relative organ weights and necropsy of the rats

Relative organ weight was calculated via the formula (organ weight/body weight) × 100%. Relative vital organ weights and microscopic changes were studied in comparison with those in the control group, and no significant changes ($p > 0.05$) were observed.

Histopathological examination of different organs of rats

Examination of liver sections from rats administered the hydroacetic extract of *O. gratissimum* leaf revealed generally preserved hepatic architecture. Hepatocytes were well-defined, though diffuse cytoplasmic vacuolation suggested mild lipid accumulation or hydropic degeneration. Hepatic sinusoids appeared slightly dilated in some areas, but the radial organization of hepatocytes remained intact. Portal tracts and central veins showed no signs of inflammation, fibrosis, or abnormal cellular infiltration, indicating the absence of severe hepatotoxicity. Minor staining artefacts were noted but did not affect interpretation, suggesting a slight adaptive metabolic response to the extract (Fig. 7A).

Liver sections from rats receiving hydroacetic extract of *V. amygdalina* leaf similarly displayed preserved lobular architecture, with hepatocytes radially arranged around central veins. Diffuse cytoplasmic vacuolation was observed, likely reflecting lipid accumulation or a mild hydropic response. Hepatic sinusoids were mildly dilated, while portal areas and central veins showed no inflammatory infiltration or fibrosis. No evidence of hepatocellular necrosis or apoptosis was detected, indicating good hepatic tolerance (Fig. 7B). Control livers exhibited normal histology (Fig. 7C).

Renal histology of rats treated with hydroacetic extract of *O. gratissimum* showed intact nephron architecture, with well-preserved glomeruli and tubules. Bowman's spaces were homogeneous, with no signs of edema or inflammation. Subtle cytoplasmic vacuolation in proximal tubules suggested reversible degeneration

or metabolic adaptation. Distal tubules appeared normal, and no necrosis or inflammatory infiltration was noted, indicating satisfactory renal tolerance (Fig. 7D).

Kidneys from rats administered hydroacetic extract of *V. amygdalina* displayed well-defined glomeruli with intact capillary structure and preserved tubular organization. Slight cytoplasmic granulation and occasional vacuolation were observed, likely reflecting normal metabolic activity or minor fixation artefacts. There was no evidence of necrosis, significant inflammatory infiltration, or basement membrane disruption, indicating no apparent renal toxicity at the administered dose (Fig. 7E). Kidney structures were comparable to those of controls (Fig. 7F).

Discussion

The results indicate that *O. gratissimum* and *V. amygdalina* leaf are rich sources of bioactive compounds, including tannins, flavonoids, saponosides, mucilages, alkaloids, and heterosides, which are of particular interest for animal health and production¹⁸. Tannins and flavonoids are recognized for their antioxidant and anti-inflammatory properties, playing crucial roles in mitigating oxidative stress and managing inflammatory conditions in livestock. Such activities are especially relevant in production systems where animals are exposed to stressors that compromise immunity and productivity. For instance, flavonoid-rich extracts could help prevent infectious diseases by modulating immune responses and reducing bacterial loads¹⁹.

Saponosides, detected only in *V. amygdalina*, have notable antimicrobial and immunomodulatory effects, potentially enhancing resistance to bacterial and parasitic infections. This property could reduce reliance on antibiotics, supporting a more sustainable and ethical approach to livestock management. Mucilages further contribute by improving digestive health.

Our analysis revealed that hydroacetic extracts of both plants contained significantly higher concentrations of total phenols, flavonoids, and condensed tannins compared to aqueous extracts. In particular, the hydroacetic extract of *V. amygdalina* exhibited elevated phenolic content (736.27 mg EAG/g ES) and condensed tannins (5.94 mg EC/g ES), suggesting strong antioxidant and antimicrobial potential⁷. Similarly, the hydroacetic extract of *O. gratissimum* showed the highest flavonoid content (37.06 mg ER/g ES), known for anti-inflammatory and cardioprotective effects, which can support intestinal and immune health^{20,21}. These findings indicate that hydroacetic extracts from both plants could be strategically used to manage oxidative stress and infections in livestock. Incorporating such extracts into animal diets may enhance resistance to infection, support digestive function, and strengthen the immune system²².

The results of the DPPH test show that ascorbic acid, used as a reference, has the highest antioxidant activity, confirming the validity of the experimental protocol. Among the plant extracts studied, the hydroacetic extracts of *V. amygdalina* and *O. gratissimum* have significantly lower IC₅₀ values than their respective aqueous extracts, indicating superior antioxidant capacity. These observations are consistent with several previous studies reporting that hydro-organic solvents improve the extraction of phenolic compounds and flavonoids, the main contributors to the antioxidant activity of medicinal plants^{23,24}. The superior activity observed for hydroacetic extracts could be explained by the increased solubility of bioactive compounds, particularly polyphenols, in solvents of intermediate polarity. *V. amygdalina* is known for its high content of sesquiterpene lactones, flavonoids, and phenolic acids, while *O. gratissimum* contains phenolic compounds, essential oils, and flavonoids with recognized antioxidant properties^{25,26}. Furthermore, the high coefficients of determination ($R^2 \geq 0.98$) obtained for all extracts reflect an excellent fit of the dose-response models and reinforce the reliability of the calculated IC₅₀ values. These data suggest that *V. amygdalina* and *O. gratissimum*, particularly in the form of hydroacetic extracts, are promising sources of natural antioxidants that can be exploited in the nutritional or veterinary fields.

The hydroacetic extracts of *O. gratissimum* and *V. amygdalina*, with low EC₅₀ values (2.40 and 3.54 respectively), could serve as effective natural alternatives for managing oxidative stress and infectious diseases in farm animals. Their low EC₅₀ values indicate that these extracts are potent at low concentrations, which may reduce costs and minimize the risk of side effects. In intensive breeding systems, incorporating these extracts could enhance animals' resistance to oxidative stress, reduce infectious diseases, and support growth^{27,28}. The use of *O. gratissimum* and *V. amygdalina* extracts could reduce the reliance on antiparasitics, particularly for preventing digestive and respiratory infections, by acting as natural antimicrobial agents. This would support sustainable animal husbandry while enhancing animal health and productivity. The in vitro results showed significant inhibition of sporulation of *E. magna* and *E. media* oocysts, with hydroacetic extracts being more effective than aqueous extracts due to their higher content of lipophilic compounds such as flavonoids, condensed tannins, and alkaloids. This observation is consistent with the work of Dagnino, et al.²⁹ and Okoye, et al.³⁰, who reported that fat-soluble bioactive compounds extracted from medicinal plants often exhibit better antiparasitic activity in vitro. At high concentrations (40 mg/ml), the hydroacetic extract of *V. amygdalina* achieved 100% inhibition of oocyst sporulation after 48 h, rivaling the efficacy of commercial coccidiostats.

These in vitro results highlight the potential of *O. gratissimum* and *V. amygdalina* extracts as natural alternatives for the management of coccidiosis in cattle. In vitro tests provide a valuable first indication, but they do not consider the complex factors present in the body, such as bioavailability, metabolism of bioactive compounds and potential interactions with the intestinal microflora^{31,32}. The progressive increase in efficacy with concentration and incubation time reflects a dose-dependent relationship, which is often observed in vitro pharmacological tests. The results also confirmed that aqueous extracts are less effective than hydroacetic extracts are, probably due to the low solubility of lipophilic compounds in water. These observations are consistent with previous work, such as that of³³, which demonstrated that organic solvent-based extracts generally exhibit better antiparasitic activity against coccidial strains. The efficacy of extracts may also be attributed to their ability to disrupt the cell membrane of oocysts or interfere with their energy metabolism, as suggested by studies on the properties of tannins and flavonoids against protozoa³⁴.

V. amygdalina and *O. gratissimum* extracts have antiparasitic effects on their bioactive compounds. Flavonoids and tannins, which are present in large quantities, are known to alter the membranes of oocysts and inhibit enzymes essential for their sporulation. Alkaloids, on the other hand, can disrupt protozoan replication mechanisms by targeting their energy metabolism. These mechanisms of action explain the high inhibition observed at increasing concentrations. The differences in efficacy between the plant species studied could be attributed to the variability in the chemical composition of the extracts, as noted by Kebede, et al.³⁵, who reported that pest control properties vary depending on the relative concentrations of flavonoids and tannins in the plant extracts. In addition, the hydroacetic extract of *V. amygdalina*, which has shown the best efficacy, seems to contain particularly potent bioactive compounds or is better extracted in organic solvents.

Extracts of *O. gratissimum* and *V. amygdalina* dose-dependently inhibited the viability of oocysts in *E. magna* and *E. media*, with a maximum performance for hydroacetic extracts, particularly *V. amygdalina* leaf hydroacetic extract (VaAc). At 1000 µg/ml, after 24 h, VaAc achieved 100% inhibition, matching the positive control, whereas the aqueous extracts showed less, but significant, efficacy. These results indicate the importance of lipophilic metabolites, such as flavonoids and alkaloids, which are better extracted by hydroacetic, for antiparasitic activity^{36,37}. The phenolic and tannic compounds present in these extracts could interfere with oocyst membranes, leading to osmotic imbalances and metabolic inhibition³⁸. Alkaloids, in particular, may target enzymes essential for oocyst development, a hypothesis supported by similar studies on other plant extracts^{39,40}. These data reinforce interest in the use of plant extracts as natural coccidiostats in poultry and rabbit farming, where coccidiosis remains a major cause of economic losses^{13,41}. The increased efficacy of hydroacetic extracts compared to aqueous extracts can be attributed to their ability to extract more fat-soluble active compounds, such as apolar flavonoids and lignans, which are less effectively extracted in aqueous solutions. This emphasizes the importance of solvent selection to optimize the antiparasitic activity of plant extracts. These findings are especially relevant in rabbit production, where coccidiosis poses significant health and economic challenges. The reversal observed between Og_Ac and Va_Aq at 2.5 µg/mL and 125 µg/mL reflects differences in time-dependent inhibitory kinetics rather than inconsistency in activity. Og_Ac exerted a stronger early effect at 24 h, whereas Va_Aq showed a delayed inhibition that became more pronounced at 48 h. As this occurred at the lowest dose, where variability is higher, interpretation relies on the overall dose- and time-dependent trend. The inhibition of *E. media* oocyst sporulation suggests that these extracts could help limit the environmental spread of infectious oocysts, reducing infectious pressure on farms and potentially replacing chemical coccidiostats, addressing concerns about drug resistance and residues. The toxicity tests on *A. salina* larvae showed no toxicity, with LC50 values well above the toxic threshold, indicating that both aqueous and hydroacetic extracts of *V. amygdalina* and *O. gratissimum* are safe for use.

These results are consistent with those of previous studies that have shown that extracts of these plants can have little or no biological effects on certain larval species, depending on the concentration used^{42,43}. The differences in the LC₅₀ values observed between the aqueous and hydroacetic extracts can be explained by the nature of the compounds extracted by each solvent. The aqueous extracts of *V. amygdalina* and *O. gratissimum* contain mainly polyphenols and flavonoids, which are known for their antioxidant effects and low toxicity^{44,45}. In contrast, hydroacetic extracts, which are rich in lipophilic compounds such as terpenes and alkaloids, could theoretically exhibit greater biological activity, but here, they are also classified as nontoxic, suggesting that the bioavailability of secondary metabolites in these extracts is not high enough to result in significant toxicity to *A. salina* larvae⁴⁶. Although the extracts were classified as nontoxic in this study, these results cannot be generalized to other species or long-term applications. Plant extracts can have toxic effects at relatively high concentrations or after prolonged exposure. Furthermore, these extracts have shown pharmacological potential in other contexts, notably in the treatment of human diseases, but their ecological impact must be taken into account before any application in the natural environment^{47,48}. In summary, these results indicate that aqueous and hydroacetic extracts of *V. amygdalina* and *O. gratissimum* have little or no toxicity to *A. salina* larvae.

The results of histological analyses of liver and kidney tissues from rats fed hydroacetic extracts of *O. gratissimum* and *V. amygdalina* leaf provide essential information on the potential metabolic and toxicological effects of these phytochemicals. Both extracts showed a general preservation of histoarchitecture in liver and kidney tissues, suggesting their relative safety at the doses administered. The diffuse cytoplasmic vacuolation observed in hepatocytes from both experimental groups indicates potential lipid accumulation or mild hydropic degeneration. These changes may reflect adaptive responses to the bioactive constituents present in *O. gratissimum* and *V. amygdalina*. Similar observations have been reported in studies evaluating the hepatoprotective and hepatomodulatory properties of phytochemicals, suggesting that mild vacuolization may represent a transient metabolic adjustment to increased lipid metabolism or oxidative stress^{49,50}. Importantly, the absence of fibrosis, necrosis or inflammatory infiltration in portal and central veins supports the idea that these extracts do not induce overt liver toxicity under the conditions studied. This finding is consistent with previous findings indicating that *O. gratissimum* possesses antioxidant and hepatoprotective properties, mainly attributed to its high polyphenol and flavonoid contents⁵¹. Renal histological analysis revealed preserved glomerular and tubular architecture, with no evidence of necrosis, inflammation or basement membrane rupture in either group. Subtle cytoplasmic vacuolation in proximal tubules may suggest reversible metabolic adjustments or mild degenerative changes related to biotransformation of plant extract constituents. These observations are consistent with the literature, which reports mild renal changes in response to bioactive compounds without significant functional alterations^{52,53}. The absence of glomerular sclerosis or interstitial fibrosis further supports the renal safety of these extracts at the doses tested. Notably, *V. amygdalina* has been reported to exert nephroprotective effects in models of oxidative stress, probably due to its richness in saponins, tannins and flavonoids^{54,55}. Although the two extracts produced similar histological results, slight differences in vacuolar changes and sinusoidal dilatation between the groups suggest variations in the bioavailability and metabolic processing of their respective phytochemical profiles. These differences could stem from the distinct chemical compositions of *O. gratissimum*

and *V. amygdalina*, which include eugenol, terpenes and flavonoids for the former and sesquiterpene lactones and saponins for the latter⁵⁶.

Limits

This study was unable to precisely identify the active compounds due to the lack of chromatographic analyses such as HPLC. In addition, anticoccidial tests were performed only in vitro, without validation under real biological conditions.

Materials and methods

Plant material collection and preparation

Fresh leaf of *O. gratissimum* and *V. amygdalina* were collected in April 2019 from organically managed market gardens at the University of Abomey-Calavi (UAC), Benin. Taxonomic identification was confirmed at the UAC Herbarium, with voucher specimens deposited under accession numbers YH524/HNB (*O. gratissimum*) and YH523/HNB (*V. amygdalina*). Leaf were rinsed with clean water, air-dried at 18 ± 1 °C for 14 days, ground into fine powder, and stored in sealed glass containers under dry, dark conditions until use.

Preparation of plant extracts

Powdered leaf of *O. gratissimum* and *V. amygdalina* were extracted by maceration. For each plant, 50 g of leaf powder was macerated in 500 mL of hydroacetic solvent (70% acetone:30% distilled water, v/v; solvent S1) at room temperature for 72 h with intermittent stirring. Filtrates were collected through Whatman No. 1 paper, and residues were remacerated until the filtrate was colorless. Combined hydroacetic extracts were concentrated under reduced pressure at 47 °C using a rotary evaporator (BUCHI R-210, Switzerland) and dried in a ventilated oven at 40 °C for 7 days to obtain dry extracts. Aqueous extracts were prepared similarly, replacing solvent S1 with distilled water, concentrating at 65 °C under reduced pressure, and oven-drying at 50 °C for 7 days.

Phytochemical screening

Qualitative phytochemical screening of powdered leaf of *O. gratissimum* and *V. amygdalina* was conducted to detect major classes of secondary metabolites. Analyses followed the standard protocols of Houghton and Raman⁵⁷, with adaptations to the facilities of the Laboratory of Biotechnologies and Animal Improvement, Faculty of Agronomic Sciences, University of Abomey-Calavi (UAC), Benin. The screening targeted alkaloids, flavonoids, tannins, saponins, sterols, terpenoids, and glycosides using characteristic colorimetric and precipitation reactions.

Determination of total phenolic content

The total phenolic content (TPC) of the plant extracts was determined using the Folin–Ciocalteu colorimetric method, following the procedure of Singleton, et al.⁵⁸, with minor modifications. A standard calibration curve was prepared using gallic acid as the reference compound. An aqueous stock solution of gallic acid (10 mg/mL) was serially diluted with distilled water to generate a range of standard concentrations. For each assay, 125 µL of plant extract (1 mg/mL in distilled water) was mixed with 625 µL of Folin–Ciocalteu reagent. After 5 min of incubation at room temperature, 500 µL of sodium carbonate solution (75 mg/mL) and 4.75 mL of distilled water were added. The mixture was thoroughly homogenized and incubated in the dark at room temperature for 30 min. Absorbance was measured at 760 nm using a BioMate 5 UV-Vis spectrophotometer (Thermo Scientific, USA), with distilled water serving as the blank. TPC was calculated from the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

Determination of Total Flavonoid Content

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method, following the procedures of Kim, et al.⁵⁹, and Zhishen, et al.⁶⁰, with minor modifications. Briefly, 500 µL of each plant extract (1 mg/mL in methanol) was mixed with 500 µL of 2% aluminum chloride (AlCl_3) solution and 3 mL of methanol. The mixture was vortexed thoroughly and incubated in the dark at room temperature for 10 min. Absorbance was measured at 415 nm using a BioMate 5 spectrophotometer (Thermo Scientific, USA), with methanol as the blank. A standard calibration curve was prepared using rutin (10 mg/mL in methanol), and TFC was expressed as milligrams of rutin equivalents per gram of extract (mg RE/g).

Determination of total condensed tannin content

Total condensed tannins were quantified using the vanillin–HCl method, following Heimler, et al.⁶¹. Briefly, 500 µL of each hydroacetic extract (1 mg/mL) was mixed with 3 mL of 4% vanillin solution, followed by the addition of 2 mL of methanol and 1.5 mL of fuming hydrochloric acid (HCl). The reaction mixture was vortexed thoroughly and incubated at room temperature for 15 min. Absorbance was measured at 500 nm using a BioMate 5 spectrophotometer (Thermo Scientific, USA), with a reagent blank as reference. A standard calibration curve was prepared using catechin under identical conditions, and results were expressed as milligrams of catechin equivalents per gram of extract (mg CE/g).

Antioxidant activity: DPPH radical scavenging assay

The antioxidant potential of *O. gratissimum* and *V. amygdalina* leaf extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, following the methods of Chokki, et al.⁶² and Dah-Nouvlessounon, et al.⁶³. This assay is based on the reduction of the purple DPPH radical to the yellow diphenylpicrylhydrazine in the presence of hydrogen-donating antioxidants. Briefly, 100 µL of hydroacetic plant extract at varying concentrations was mixed with 1.9 mL of freshly prepared DPPH solution (3.10^{-4} M

in methanol), yielding a total reaction volume of 2 mL. Mixtures were vortexed and incubated in the dark at room temperature. Absorbance at 517 nm was recorded at 2-minute intervals using a BioMate 5 UV-Vis spectrophotometer (Thermo Scientific, USA) until a steady state was reached, typically within 30 min. Samples with absorbance values outside the linear range of the standard curve were appropriately diluted in methanol, as recommended by Schmeda-Hirschmann, et al.⁶⁴. Ascorbic acid was used as a positive control and evaluated over the same concentration range as the plant extracts (1–1000 µg/mL) to ensure direct comparability of antioxidant activity under identical experimental conditions.

The percentage of DPPH radical scavenging activity was calculated as:

$$I (\%) = \frac{Abs\ control - Abs\ compound}{Abs\ control} * 100$$

The extract concentration required to achieve 50% inhibition (IC₅₀) was determined from the plot of percentage inhibition versus extract concentration.

The IC₅₀ values were determined using a four-parameter log-logistic model implemented in the drc package in R 4.5.1.

***In vitro* anticoccidial activity of *O. gratissimum* and *V. amygdalina* leaf extracts**

Preparation of E. magna and E. media Cultures

A 2.5% (w/v) potassium dichromate (K₂Cr₂O₇) solution was prepared by dissolving 2.5 g of K₂Cr₂O₇ in 100 mL of distilled water and stored at room temperature for use in oocyst preservation and extract dilution. Sporulated oocysts of *E. magna* and *E. media* were obtained from field isolates collected from the jejunum and ileum of three naturally infected rabbits, confirmed using the flotation technique. The animals were sourced from independent local breeders.

Oocysts were recovered, washed, and concentrated using standard flotation methods, then suspended in 2.5% K₂Cr₂O₇ and stored at 4 °C until use. For long-term maintenance, periodic passages were performed in healthy New Zealand White rabbits housed at the Laboratory of Biotechnology and Animal Improvement, Faculty of Agronomic Sciences, University of Abomey-Calavi, Benin.

In vitro Oocysticidal Assay

The oocysticidal activity of the plant extracts was assessed in vitro using Petri dishes. Each well received 2 mL of extract at five concentrations (2.5, 5, 10, 20, and 40 mg/mL) prepared in 2.5% K₂Cr₂O₇. A standardized inoculum of unsporulated oocysts was added to each well. Plates were incubated at 28 °C for 24 h and 48 h. A 2.5% phenol solution served as the positive control.

Sporulation rates were determined microscopically by counting 100 oocysts per replicate. The percentage of sporulation inhibition was calculated as:

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

In vitro Anti-sporozoidal Assay

Sporozoites were released from the sporulated oocysts through excystation. Briefly, oocysts stored in K₂Cr₂O₇ were washed repeatedly with Hank's Balanced Salt Solution (HBSS; pH 7.2) to remove residual dichromate. The washed oocysts were incubated in a water bath at 41 °C with constant agitation for 60 min. Following incubation, the mixture was centrifuged at 3,000–5,000 × g for 10 min. The sporozoite-rich supernatant was collected, washed again with HBSS, and enumerated using a McMaster counting chamber. To assess the anti-sporozoidal activity, 2 mL of each plant extract (250, 500, 750, and 1000 µg/mL) was added to wells of Petri dishes containing a standardized number of sporozoites. Amprocox® (a commercial anticoccidial drug) was used as a positive control. After incubation at 28 °C for 12 h and 24 h, the number of viable and non-viable sporozoites was counted microscopically⁶⁵. Viability inhibition was calculated using the following formula:

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

Larval Cytotoxicity Assay of Aqueous and hydroacetic Extracts of O. gratissimum and V. amygdalina

The cytotoxic potential of the aqueous and hydroacetic leaf extracts of *O. gratissimum* and *V. amygdalina* was assessed using the brine shrimp (*A. salina*) lethality bioassay, following the protocol described by Adoho, et al.⁶⁶, with adaptations for laboratory conditions.

Hatching of A. salina Larvae

Ten milligrams of *A. salina* cysts were incubated in 1 L of artificial seawater (prepared by dissolving 38 g of marine salt in 1 L of distilled water) under continuous aeration and illumination at 28 °C for 48 h. After hatching, the nauplii were collected and used for the bioassay.

Cytotoxicity Assay

Stock solutions of each plant extract were prepared at 20 mg/mL in the appropriate solvent (distilled water for aqueous extracts and 70% acetone for acetone extracts), and serial dilutions were performed to obtain a range of test concentrations. For each concentration, 1 mL of extract solution was combined with 1 mL of seawater containing 16 live *A. salina* nauplii in sterile 24-well plates. A control group containing only seawater and larvae was included under identical conditions. Each treatment and control was performed in triplicate.

After 24 h of incubation under continuous agitation at room temperature, larval mortality was assessed using an optical microscope. The percentage mortality was calculated, and the dose–response relationship was plotted as the number of surviving larvae against the logarithm of extract concentration. Larvae of *A. salina* were used as a preliminary toxicity screening model due to their simplicity, rapid response, low cost, and widespread use for predicting general cytotoxicity of natural products prior to in vivo evaluation.

Determination of LC_{50}

The median lethal concentration (LC_{50}) was calculated using linear regression of the log-transformed concentrations and corresponding mortality percentages. The toxicity of the extracts was classified based on the criteria proposed by Ugwah-Oguejiofor, et al.⁶⁵:

- $LC_{50} \geq 0.1$ mg/mL: Non-toxic.
- 0.1 mg/mL $> LC_{50} \geq 0.050$ mg/mL: Low toxicity.
- 0.050 mg/mL $> LC_{50} \geq 0.010$ mg/mL: Moderate toxicity.
- $LC_{50} < 0.010$ mg/mL: High toxicity.

This bioassay provided an initial evaluation of the safety profile of the plant extracts and informed subsequent in vivo studies.

Acute oral toxicity of hydroacetic extracts of *O. gratissimum* and *V. amygdalina* leaf

Ethical Approval for Animal Experiments

The study was approved by the Research and Ethics Committee of the National Agricultural University of Benin (N° 143–2018/President-CER/SA). Throughout the study, measures were taken to ensure that animal welfare was respected during experimentation. The experiments were carried out by trained and certified researchers in compliance with current regulations on the use of animals for scientific purposes (European Directive 2010/63/EU/IACUC). The authors confirm full compliance with the ARRIVE guidelines 2.0 for transparent reporting of in vivo experiments.

Animal materials

Nine female albino Wistar rats, aged at least three months and weighing 150–200 g, were used for the acute oral toxicity study. All animals were nulliparous and nonpregnant, and were obtained from the Institute of Applied Biomedical Sciences, University of Abomey-Calavi, Benin. Rats were randomly housed in groups of three in stainless steel-caged units and allowed to acclimate for 14 days at the animal facility of the Zootechnical Research and Livestock Systems Unit (URZoSE), National University of Agriculture. Animals had ad libitum access to food and water. Environmental conditions were maintained at 23 ± 2 °C with a relative humidity of $60 \pm 10\%$ and a 12-hour light/dark cycle.

Study design

Acute oral toxicity of the hydroacetic extracts of *O. gratissimum* and *V. amygdalina* was evaluated following OECD guideline No. 423⁶⁷. The nine rats were randomly assigned into three groups of three animals each, with similar mean body weights: Group 1 (control), Group 2 (hydroacetic extract of *O. gratissimum*), and Group 3 (hydroacetic extract of *V. amygdalina*). All rats underwent a 12-hour fast prior to administration, with water provided ad libitum, and individual body weights were recorded.

Rats in Groups 2 and 3 received a single oral dose of the respective hydroacetic extract dissolved in 1 mL of distilled water via gavage, while the control group received 1 mL of distilled water. Animals were observed individually for the first 30 min, then continuously over the first 24 h, and subsequently daily for 14 days for signs of macroscopic toxicity, including convulsions, agitation, moribund state, severe distress, or mortality. Body weights were measured on days 1, 7, and 14. At the end of the 14-day period, Wistar rats were anesthetized by intraperitoneal injection of sodium thiopental at a dose of 40 mg·kg⁻¹, in accordance with established protocols for anesthetic induction in this species. Once deep anesthesia was confirmed (absence of corneal and paw reflexes), the animals were euthanized by cervical dislocation. Predefined humane euthanasia criteria were applied throughout the toxicity study, including weight loss greater than 20%, severe respiratory distress, prolonged inability to feed or move, and signs of unrelieved pain. These criteria allowed for early euthanasia in cases of obvious suffering, in accordance with institutional ethical recommendations.

Major organs such as the brain, liver, spleen, heart, and kidneys were excised, weighed, and macroscopically examined for lesions or abnormalities. Relative organ weights were calculated as a percentage of total body weight. Organ samples were fixed in 10% neutral buffered formalin for subsequent histopathological analysis.

All procedures, including gavage administration and post-treatment monitoring, were performed by trained personnel in accordance with institutional and regulatory animal care guidelines.

Biochemical and haematological analysis

On day 14 of treatment, 1 mL of blood was collected from each rat via puncture of the retro-orbital plexus using heparinized capillary tubes, into both plain and EDTA-coated tubes. Blood in the plain tubes was centrifuged

at 2,500 rpm for 5 min at 4 °C to obtain serum, which was analyzed using commercial kits for key biochemical biomarkers: blood glucose (GOD-PAP, Biolabo, Ref. 7409), creatinine (Biolabo, Ref. 0107), urea (Biolabo, Ref. 0021), aspartate aminotransferase (AST, Biolabo, Ref. 0025), alanine aminotransferase (ALT, Biolabo, Ref. 0027), alkaline phosphatase (ALP, Biolabo, Ref. 80014), and gamma-glutamyl transferase (γ -GT, Biolabo, Ref. 81310). Absorbance readings were obtained using an SP-350-BIO spectrometer (COLE-PARMER, formerly GenovaPlus, JENWAY).

Blood collected in EDTA-coated tubes was used for hematological analysis, including red blood cell (RBC) and white blood cell (WBC) counts, hemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT) count. Measurements were performed using an automated Beckman Coulter Ac hematology analyzer⁴³.

Histological examination

Following euthanasia, liver and kidney tissues were excised and processed into paraffin blocks using labeled tissue processing cassettes. Samples were dehydrated through a graded ethanol series (70%, 80%, 90%, and absolute alcohol), followed by two additional changes of absolute alcohol and three changes of xylene. Tissues were then infiltrated and embedded in paraffin.

Sections of 4 μ m thickness were cut from each paraffin block, mounted onto microscope slides, and stained with hematoxylin and eosin (H&E) according to Ugwah-Oguejiofor, et al.⁶⁵. Stained sections were examined under an Olympus microscope for morphological changes, and representative images were captured for documentation.

Statistical analysis

Experimental data were systematically organized using Excel 2019 and expressed as mean $\pm$ standard deviation (SD) of three replicates. One-way analysis of variance (ANOVA) was performed to assess differences between groups, followed by the Waller-Duncan post hoc test, with $p < 0.05$ considered statistically significant. Statistical analyses were conducted using R software (version 4.4.2). IC₅₀ values for extracts and key bioactive compounds demonstrating strong anticoccidial activity, as well as LC₅₀ values for extract safety, were determined via regression analysis. Graphical representations and additional statistical evaluations were generated using GraphPad Prism® (version 10.4.1).

Conclusion

This study evaluated the antioxidant and anticoccidial properties of aqueous and hydroacetic extracts of *V. amygdalina* and *O. gratissimum* for their potential to manage coccidial infections in rabbits. Antioxidant analyses revealed significant activity attributed to the presence of bioactive compounds such as flavonoids and phenols, which were particularly concentrated in the hydroacetic extracts. The hydroacetic extracts also markedly inhibited the sporulation of *E. magna* and *E. media* oocysts, suggesting greater efficacy than aqueous extracts. In vitro and in vivo toxicity tests confirmed the relative safety of the extracts, with no evidence of larval toxicity or acute oral toxicity. Histological examination of the target organs revealed no toxicity at a dose of 2000 mg/kg. Among the plants studied, *V. amygdalina* stood out for its superior anticoccidial properties. These results highlight the potential of these plants as natural and sustainable alternatives to conventional treatments for coccidiosis while offering additional benefits linked to their antioxidant properties.

Perspectives

Future research should incorporate molecular analyses (e.g., HPLC, LC-MS) to characterize active constituents and conduct in vivo trials to validate the efficacy and safety of these extracts under practical farming conditions.

Data availability

The raw data for the current study are available from the corresponding author upon reasonable request.

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Declarations

Competing interests

The authors declare no competing interests.

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