Comparative study on phyto-constituents, antioxidants and biological activity of Daniellia oliveri leaf and stem bark extracts.

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Abstract

Daniellia oliveri is an ethnomedicinal plant traditionally used for the management of several health conditions. This study comparatively evaluated the phytochemical composition, anti-nutritional factors, antioxidant, anthelmintic and antiemetic activities of methanol and n-hexane extracts of the leaves and stem bark of *Daniellia oliveri*. Leaf and stem bark samples were extracted separately with methanol and n-hexane and evaluated using standard phytochemical, antioxidant, anthelmintic and antiemetic assays. The methanol stem bark extract contained the highest concentrations of total phenolics (161.12 mg GAE/g), flavonoids (51.05 mg QE/g), tannins (20.15 mg CE/g), and saponins (36.96 mg DE/g). In contrast, alkaloids were highest in the n-hexane leaf extract (10.07 %). In the DPPH assay, MS exhibited the lowest IC_{50} among the extracts (194.98), whereas the n-hexane leaf extract had the highest IC_{50} (588.84); vitamin C showed an IC_{50} of 64.57. In the *in-vitro* anthelmintic assay, the methanol leaf extract produced the shortest paralysis time among the plant extracts, with 15.55 min at 50 mg/mL, compared with 12.50 min for piperazine citrate at 10 mg/mL. In the chick antiemetic model, the n-hexane stem bark extract produced 60.25 % inhibition of retching at 150 mg/kg, followed by the methanol stem bark extract (52.32 %). These findings demonstrate that the biological activities of *Daniellia oliveri* vary according to plant part and extraction solvent. The results provide preliminary experimental evidence supporting further investigation of *Daniellia oliveri* as a source of bioactive constituents with antioxidant, anthelmintic and antiemetic potential. However, identification of the active compounds, mechanistic studies, comprehensive toxicity evaluation and pharmacological validation are required before therapeutic applications can be proposed.

Keywords: Anthelmintic, Antiemetic, Antioxidant, *Daniellia oliveri*, Phytochemical, Plant extracts.

1. Introduction

Medicinal plants are defined by their ability to provide therapeutic benefits or exert favorable pharmacological influences on human and animal physiology. Beyond serving fundamental needs such as nutrition and shelter, vegetation has historically functioned as the primary source of herbal remedies. These plants synthesize a diverse array of secondary metabolites biologically active compounds that serve as critical precursors for modern drug development and various therapeutic interventions (Adeleye, 2014).

In contemporary clinical settings, nausea and emesis remain significant challenges, particularly as adverse effects of chemotherapy. These symptoms often lead patients to delay or even abandon life-saving treatments (Jordan *et al*., 2007; Aseeri *et al*., 2013). Although progress has been made in managing acute and delayed emesis, there is a persistent demand for more effective antiemetic agents (Davis, 2016). Currently, effective relief is achieved in only 70 to 80 % of patients undergoing highly emetogenic cytotoxic therapy. This variability in response is often attributed to genetic polymorphisms in drug receptors, age, gender, and the specific emetogenic profile of the treatment. Consequently, the search for novel, plant-derived antiemetic agents remain a priority in supportive oncology care (Perwitasari *et al*., 2011).

Similarly, helminthiasis or parasitic worm infestation presents a global public health crisis, impacting not only human health but also livestock and agricultural productivity. The rising

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prevalence of anthelmintic resistance among nematodes, coupled with the side effects associated with synthetic deworming drugs, has intensified the global effort to identify botanical alternatives with potent anthelmintic properties (Akhtar *et al*., 2000; Jackson and Coop, 2000).

Daniellia oliveri (Rolfe) Hutch and Dalziel, a member of the Caesalpiniaceae family, is a significant ethnomedicinal tree native to Africa and the Amazonian regions (Atolani and Olatunji, 2016). Known as "emi ya" in Southwest Nigeria, this underutilized species holds substantial economic and medicinal promise. Traditional applications are diverse: in Gambia, its aromatic resins serve as mosquito repellents, while bark decoctions are employed to manage gastrointestinal parasites (Djoueche *et al*., 2011). In Southeast Nigeria, root combinations of *Daniellia oliveri* and *Sarcocephalus latifolius* are used to treat hyperglycemia (Iwueke and Nwodo, 2008).

Previous research on *Daniellia oliveri* leaves has highlighted their antimicrobial, antioxidant, antityphoid, and antidiarrheal activities, as well as their effects on skeletal muscle and cardiovascular indices (Ahmadu e*t al*., 2004; Musa *et al*., 2010). The stem bark is recognized for producing exudates rich in oleoresins and terpenoids, which are utilized for wound healing and as cosmetic anti-wrinkle agents (Igoli *et al*., 2005). Furthermore, compounds such as daniellic acid and diterpene lactones have been isolated from its wood (Olatunji, 2000; Atolani and Olatunji, 2016).

Despite these reports, an important gap remains. Previous investigations have generally examined individual biological activities or plant parts, whereas comparatively limited information is available on the relationship between the phytochemical composition and the antioxidant, anthelmintic and antiemetic activities of *Daniellia oliveri* leaves and stem bark extracted using solvents of different polarity within the same experimental framework. In particular, direct comparative information on methanol and n-hexane extracts of both plant parts, together with quantitative phytochemical characterization and evaluation in the same study, remains limited. The present study therefore aimed to compare the phytochemical composition, selected anti-nutritional factors, antioxidant capacity, anthelmintic activity and antiemetic activity of methanol and n-hexane extracts of *Daniellia oliveri* leaves and stem bark.

The study was designed to determine whether differences in biological activity correspond with differences in the measured phytochemical profiles. However, because the study did not isolate individual compounds or directly test purified constituents, associations between phytochemical classes and biological activities are interpreted as correlations or plausible explanations rather than evidence of causation.

2. Materials and methods

2.1. Plant collection and preparation of extracts

Stem bark and fresh leaves of *Daniellia oliveri* were collected from a farm at Anyigba (7º 28’ 51.39” N and 7º 11’ 14.86 E), Kogi State, Nigeria in August 2021. The plant material was authenticated by a botanist in the Department of Plant Science and Biotechnology, Prince Abubakar Audu University, Anyigba and a Voucher Specimen Number (PAAU-PT-B-020) was deposited in the Department of Plant Science and Biotechnology Herbarium. The leaves and stem bark were cleaned, shade-dried in the laboratory. The leaves were pulverized and blended into fine powder using an electric blender and the stem bark were pulverized and sieved into a fine powder.

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Powdered leaves (340 g) and stem bark (240 g) were extracted separately by continuous cold maceration in methanol or n-hexane at a 1:5 w/v powdered sample-to-solvent ratio for a period of 48 hours with agitation at interval. The mixtures were filtered through Whatman filter paper and concentrated under reduced pressure at 45°C until constant weights were obtained. Percentage (%) extraction was calculated as:

$$\% \text{ yield} = \frac{\text{Weight of extract (g)}}{\text{Weight of powdered sample (g)}} \text{ x } 100 \quad \text{- - - - (1)}$$

The crude extracts were stored in airtight container at refrigerated temperature until analysis.

2.2. Quantitative phytochemical evaluation

The phytochemical contents of *Daniellia oliveri* leaf and stem bark extracts were quantified using colorimetric and gravimetric techniques. Specifically, total tannin content was evaluated via the vanillin-HCl method as modified by He *et al*. (2022), while the total phenolic and flavonoid contents were determined using the Folin-Ciocalteu assay described by Arogba (2014) and the chloride colorimetric method (Al-Rimawi *et al*., 2020), respectively. Additionally, saponin concentrations were estimated following the procedures described by Oloyede *et al*. (2020). Alkaloid and cardiac glycoside levels were quantified according to the protocol described by Sreevidya and Mehrotra (2003).

2.3. Analysis of anti-nutritional factors (phytotoxins)

The anti-nutritional composition of extracts was evaluated by quantifying phytic acid, cyanide, oxalate, and nitrate contents. Phytate levels were estimated using the Iron (III) chloride ($FeCl_3$) titration method (Gupta *et al*., 2021), while cyanide concentration was determined via the alkaline picture (Onwuka, 2018). Total oxalate content was quantified by potassium permanganate ($KMnO_4$) titration according to the protocols described by Onwuka (2018) and Adeniyi *et al*. (2019). Finally, nitrate concentration was determined using the standard Kjeldahl procedure (ISO 20483:2013; Aktas *et al*., 2022).

2.4. *In vitro* antioxidant analysis

The antioxidant potential of the extracts was evaluated using two different techniques; 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP), following the method of Omada and Arogba (2019).

2.4. *In vitro* anthelmintic assay

This was conducted following the protocol described by Das *et al.* (2023) with minor modifications. Earthworms (*Pheretima posthuma*) of similar size were harvested from moist soil at Ogane-aji riverbank, Anyigba, Kogi State, and washed with normal saline. A total of 14 groups, 4 earthworms per group were established. Methanol and n-hexane extracts of *Daniellia oliveri* (leaf and stem bark) were dissolved in 2% v/v Tween 80 and adjusted with normal saline to yield concentrations of 10, 20, and 50 mg/mL. The worms were transferred into 10 mL of the respective formulations:

Group 1 (control) received 10 mL of 2% v/v Tween 80 in normal saline, to control for solvent effects.

Group 2 (standard) received Piperazine citrate (10 mg/mL) (Verma *et al*., 2020).

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Groups 3 - 14 (test groups) received test extracts at concentrations of 10, 20, and 50 mg/mL (Table 4).
The time taken for paralysis (loss of movement after vigorous shaking) of the earthworms was observed and recorded for each group.

2.5. *In vivo* antiemetic assay

The antiemetic effect was evaluated using the copper sulfate-induced emesis model in chicks (Silva *et al*., 2021; Hussain *et al*., 2023). Experimental protocol was approved by the Department of Biochemistry, Prince Abubakar Audu University Postgraduate Studies Board, at a proposal seminar defense organized by the department for Post Graduate Students in July 2021. A day-old AGRITED Ross 308 broiler chicks (34 - 52 g) were obtained from Agricultural International Technology and Trade Nigeria Limited (AGRITED), Ibadan, Oyo state, Nigeria. The chicks were housed at the Experimental Animal House of Department of Biochemistry, Prince Abubakar Audu University, under controlled temperature (32 - 35 °C) and 24 hours light with access to commercial Top-feed Broiler Starter and water *ad-libitum* for a period of 3-day. On the 4th day, twenty-four (24) chicks were randomly divided into 6 groups, 4 chicks per group and administered treatments orally as follows:
Group 1 (control) received distilled water (10 mL/kg).
Group 2 (standard) received Metoclopramide (150 mg/kg).
Group 3 – 6 received plant extracts (150 mg/kg) each, as shown in Table 5.
All treatment groups; control, standard and plant extracts received equal dosing volume of 10 mL/kg.
Ten minutes post-treatment, copper sulfate (50 mg/kg) was administered to induce emesis.
Antiemetic activity was assessed by counting the number of retches over a 10-minute period. The percentage inhibition of retching relative to the negative control group was calculated using the formula:

$$\% \text{ Inhibition of retching} = \frac{\text{Control} - \text{Test}}{\text{Control}} \text{ x } 100 \quad - \quad - \quad - \quad - \quad - \quad (2)$$

2.6. Data analysis

Data was analyzed using SPSS Version 26.0 (IBM Corp., Armonk, NY, USA) and presented as Mean ± Standard Error of the Mean (SEM). Group means were compared using One-Way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons ($p < 0.05$). IC_{50} values were derived using non-linear regression analysis of dose-response curves. Sample sizes (n = 3 for phytochemical, phototoxins and antioxidant assays and n = 4 for biological assays) were selected based on established screening protocols in ethnopractice literature and to minimize animal usage (3Rs principle); formal a priori power calculations were not performed.

3. Results

3.1. The extraction yields were as follows: Methanol stem bark extract (MS) = 12.8 %; methanol leaf extract (ML) = 8.5 %; n-hexane stem bark extract (NS) = 1.9 % and n-hexane leaf extract (NL) = 6.1 %.

3.2. Phytochemical composition of *Daniellia oleiveri* leaf and stem bark extracts

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Table 1 shows that tannins, total phenols, flavonoids, cardiac glycosides, and saponins were significantly higher ($p < 0.05$) in the stem bark extracts than in the leaf extracts, with the methanol stem bark extract recording the highest values. In contrast, alkaloid content was significantly highest in the n-hexane leaf extract. No significant differences ($p > 0.05$) were observed between the methanol and n-hexane stem bark extracts or between the methanol and n-hexane leaf extracts for tannins, total phenols, and cardiac glycosides.

Table 1. Phytochemical composition of *Daniellia oliveri* leaf and stem bark extracts

Parameters	MS	NS	ML	NL	SEM
Tannins (mg CE/g)	20.15 ± 0.49^{b}	19.21 ± 0.02^{b}	6.66 ± 0.01^{a}	5.28 ± 0.01^{a}	3.97
Total phenol (mg GAE/g)	161.12 ± 0.06^{b}	137.12 ± 0.05^{b}	67.67 ± 0.10^{a}	44.51 ± 0.19^{a}	27.71
Flavonoid (mg QE/g)	51.05 ± 0.03^{c}	37.74 ± 0.08^{b}	13.61 ± 0.03^{a}	11.56 ± 0.07^{a}	9.59
Alkaloids (%)	1.90 ± 0.31^{a}	5.23 ± 0.15^{b}	6.17 ± 0.73^{b}	10.07 ± 0.07^{c}	1.68
Cardiac gly. (mg SS/g)	3.92 ± 0.01^{b}	3.20 ± 0.01^{b}	1.50 ± 0.01^{a}	0.85 ± 0.06^{a}	0.72
Saponins (mg DE/g)	36.96 ± 0.01^{c}	24.13 ± 0.02^{b}	7.92 ± 0.01^{a}	5.67 ± 0.01^{a}	7.35

Values with the same superscripts on the same row are not significantly different at $p > 0.05$. Results were presented as mean ± SEM at three replicates, where; MS= methanol extract of stem bark, NS= n-hexane extract of stem bark, ML= methanol extract of leaf, NL= n-hexane extract of leaf. CE = catechin equivalent, GA = gallic acid equivalent, QE = quercetin equivalent

3.3. Phytotoxins composition of *Daniellia oleiveri* leaf and stem bark extracts

As shown in Table 2, phytate and nitrate contents were significantly higher ($p < 0.05$) in the n-hexane extracts than in the methanol extracts. Cyanide content was highest in the methanol stem bark extract and lowest in the n-hexane leaf extract, while oxalate content was significantly higher in the leaf extracts than in the stem bark extracts ($p < 0.05$).

Table 2. Phytotoxins composition of *Daniellia oliveri* leaf and stem bark extracts.

Parameters	MS	NS	ML	NL	SEM
Phytate (mg/g)	62.30 ± 1.49^{a}	102.80 ± 0.50^{b}	82.50 ± 0.50^{a}	114.82 ± 3.74^{b}	11.55
Cyanide (µg/g)	55.13 ± 0.01^{d}	41.64 ± 0.04^{c}	24.21 ± 0.01^{b}	15.22 ± 0.02^{a}	8.91
Oxalate (mg/g)	3.72 ± 0.01^{a}	4.50 ± 0.01^{a}	9.36 ± 0.03^{c}	5.82 ± 0.05^{b}	1.25
Nitrate (%)	1.82 ± 0.01^{b}	2.84 ± 0.01^{c}	0.68 ± 0.01^{a}	2.94 ± 0.02^{c}	0.53

Values with the same superscripts on the same row are not significantly different at $p > 0.05$. Results were presented as mean ± SEM at three replicates, where; MS= methanol extract of stem bark, NS= n-hexane extract of stem bark, ML= methanol extract of leaf, NL= n-hexane extract of leaf.

3.3. Antioxidant activity of *Daniellia oleiveri* leaf and stem bark extracts

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As shown in Table 3(a, b), vitamin C exhibited the highest antioxidant activity with the lowest IC_{50}. Among the plant extracts, the methanol stem bark extract displayed the strongest antioxidant activity, as evidenced by the lowest IC_{50} value. The n-hexane leaf extract exhibited the weakest antioxidant activity with the highest IC_{50} value (588.84 µg/mL). Statistically, the order of antioxidant activity among the extracts was MS > ML = NS > NL ($p < 0.05$). Similarly, the ferric reducing antioxidant power (FRAP) assay (Table 3b) results generally supported the antioxidant trend observed in the DPPH assay. Higher FRAP denoted higher antioxidant activity.

Table 3a. Percentage Inhibition of DPPH in varies concentrations of *Daniellia oliveri* leaf and stem bark extracts

Conc. (µg/mL)	MS (%)	NS (%)	ML (%)	NL (%)	Vitamin C (%)	SEM
3200	92.0	73.3	76.3	74.3	95.2	
1600	87.9	66.4	72.9	62.9	84.6	
800	70.6	59.3	62.0	56.4	80.1	
400	62.4	56.7	59.3	47.7	72.5	
200	50.3	47.4	47.7	39.5	61.2	
100	38.8	31.9	31.9	32.2	55.2	
IC_{50}	194.98^{b}	316.23^{c}	288.40^{c}	588.84^{d}	64.57^{a}	86.56

Values with the same superscripts in the same row are not significantly different at $p > 0.05$. Results were presented as mean ± SEM at three replicates, where; MS= methanol extract of stem bark, NS= n-hexane extract of stem bark, ML= methanol extract of leaf, NL= n-hexane extract of leaf.

The percentage (%) inhibition was plotted against the Log concentration of the extracts (µg/mL) to determine the IC_{50}, as Log-^{1}x (µg/mL) from the equation: $y = mx + c$ when $y = 50$.

Table 3b. FRAP value of *Daniellia oliveri* leaf and stem bark extracts

Extracts	FRAP value	(Vitamin C eq. in mM)
MS	1.72 ± 0.40^{c}	24.42 ± 0.75^{c}
NS	1.45 ± 0.27^{b}	20.59 ± 0.44^{b}
ML	1.47 ± 0.07^{b}	20.87 ± 0.38^{b}
NL	1.32 ± 0.21^{a}	18.74 ± 0.46^{a}
Vitamin C	2.00 ± 0.00^{d}	28.38 ± 0.00^{d}
SEM	0.12	1.71

Values with the same superscripts on the same column are not significantly different at $p > 0.05$. Results were presented as mean ± SEM at three replicates, where; MS = methanol extract of stem, NS = n-hexane extract of stem bark, ML = methanol extract of leaf, NL = n-hexane extract of leaf.

3.5. *In vitro* anthelmintic activity of *Daniellia oleiveri* leaf and stem bark extracts

Table 4 shows that Piperazine citrate displayed the highest anthelmintic activity. Among the extracts, the methanol leaf extract exhibited the greatest activity, while the methanol stem bark extract showed the least activity. Increasing extract concentration generally reduces the time of paralysis, indicating dose-dependent anthelmintic activity. However, no significant difference (p

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> 0.05) was observed between the 20 and 50 mg/mL concentrations of the methanol stem bark extract, between the 10 and 20 mg/mL concentrations of the methanol leaf extract, and among the concentrations of the n-hexane leaf extract.

Table 4. *In vitro* anthelmintic activity of *Daniellia oliveri* leaf and stem bark extracts.

Group	Sample	Concentration (mg/ml)	Time of paralysis (min.)
1	Control	0.00	Nil
2	Piperazine citrate	10.00	12.50 ± 0.87^{a}
3	MS	10.00	37.26 ± 0.63^{g}
4	MS	20.00	33.75 ± 0.25^{f}
5	MS	50.00	31.72 ± 0.75^{f}
6	ML	10.00	17.78 ± 0.48^{c}
7	ML	20.00	17.00 ± 0.71^{bc}
8	ML	50.00	15.55 ± 0.48^{b}
9	NS	10.00	25.15 ± 0.63^{e}
10	NS	20.00	24.50 ± 0.65^{e}
11	NS	50.00	21.10 ± 0.96^{d}
12	NL	10.00	22.25 ± 0.65^{d}
13	NL	20.00	21.40 ± 0.96^{d}
14	NL	50.00	19.00 ± 065^{cd}
	SEM		2.03

Values with the same superscripts on the same column are not significantly different at $p > 0.05$. Results were presented as mean ± SEM at four replicates, where; MS= methanol extract of stem bark, NS= n-hexane extract of stem bark, ML= methanol extract of leaf, NL= n-hexane extract of leaf.

3.6. Antiemetic effects of *Daniellia oleiveri* leaf and stem bark extracts

Table As shown in Table 5, Metoclopramide produced the highest antiemetic activity. Among the extracts, the n-hexane stem bark extract showed the highest antiemetic effect, followed by the methanol stem bark extract, whereas both leaf extracts exhibited significantly lower activities. No significant difference ($p > 0.05$) was observed between the methanol and n-hexane stem bark extracts or between the methanol and n-hexane leaf extracts.

Table 5. Antiemetic effect of *Daniellia oliveri* leaf and stem bark extracts in copper sulfate induced emesis in chicks

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Group	Dose	No. of retches	% Inhibition of retching
1	Control (10 mL/kg)	80.50 ± 0.20^{d}	0.00 ± 0.00^{a}
2	Metoclopramide (150 mg/kg)	25.00 ± 1.83^{a}	68.94 ± 1.20^{d}
3	ML (150 mg/kg)	71.88 ± 0.88^{cd}	10.71 ± 0.47^{b}
4	NL (150 mg/kg)	69.00 ± 0.54^{c}	14.29 ± 0.21^{b}
5	MS (150 mg/kg)	38.38 ± 2.21^{b}	52.32 ± 1.14^{c}
6	NS (150 mg/kg)	32.00 ± 1.02^{ab}	60.25 ± 1.09^{cd}
SEM		9.67	12.06

Values with the same superscripts on the same column are not significantly different at $p > 0.05$. Results were presented as mean ± SEM at four replicates, where; MS= methanol extract of stem bark, NS= n-hexane extract of stem bark, ML= methanol extract of leaf, NL= n-hexane extract of leaf.

5. Discussion

5.1. Phytochemical composition of *Daniellia oliveri* leaf and stem bark extracts

Quantitative phytochemical analysis of *Daniellia oliveri* revealed marked variations in the distribution of the compounds between stem bark and leaf extracts, as well as between extraction solvents, demonstrating the influence of both plant part and solvent polarity on phytochemical recovery.

The significantly higher concentrations of tannins, total phenolics, flavonoids, cardiac glycosides, and saponins in the stem bark compared to corresponding leaf extracts (Table 1) indicate that the stem bark serves as a richer reservoir of these pharmacologically active compounds. Although methanol generally extracted higher quantities of these phytochemicals than n-hexane, no significant differences were observed between methanol and n-hexane stem bark extracts for tannins, total phenolics, and cardiac glycosides. This suggests that the compounds are relatively abundant in the stem bark irrespective of solvent polarity. Similarly, comparable concentrations in the methanol and n-hexane leaf extracts indicate a relatively uniform distribution of these constituents within the leaves.

The relatively high tannin content detected in the stem bark is of considerable pharmacological importance. Tannins are high-molecular-weight polyphenolic compounds capable of forming stable complexes with proteins, thereby disrupting microbial enzymes and cellular proteins, which underlies their antimicrobial, antiviral, and antiparasitic activities (Silanikove *et al*., 2001). Beyond these effects, tannins possess potent antioxidant properties by scavenging reactive oxygen species and preventing oxidative damage to cellular macromolecules (Rajakumari *et al*., 2020). Consequently, elevated tannin concentrations could explain the superior biological activities demonstrated by the stem bark extracts in this study.

A similar trend occurred for total phenolic and flavonoids, with stem bark extracts exhibiting significantly greater phenolic content than leaf extracts. Phenolic compounds and flavonoids are recognized as major contributors to the antioxidant capacity of medicinal plants due to their ability to donate hydrogen atoms or electrons to neutralize free radicals and terminate oxidative chain

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reactions (Adeleye *et al*., 2014; Afuwape *et al*., 2025). Their antioxidant effects protect essential biomolecules like DNA, proteins, and membrane lipids from oxidative damage, reducing the risk of several chronic disorders associated with oxidative stress. Additionally, phenolic compounds exhibit antimicrobial and anti-inflammatory properties that enhance their medicinal value (Alagbe, 2020), providing a plausible biochemical explanation for the superior performance of the stem bark in subsequent antioxidant assays as well as the enhanced pharmacological activities of this plant extract.

In contrast, alkaloid distribution differed markedly. The n-hexane leaf extract exhibited the highest alkaloid content, while the methanol stem bark extract contained the lowest concentration. This variation suggests that certain alkaloids present in this plant possess relatively non-polar characteristics, making them more amenable to extraction with n-hexane. Although alkaloids were more abundant in the leaf extracts, the superior biological activities demonstrated by the stem bark indicate that the observed pharmacological effects may depend more strongly on the synergistic interaction of phenolics, flavonoids, tannins, saponins, and cardiac glycosides than on alkaloid concentration alone.

Cardiac glycosides and saponins also occurred in significantly higher concentrations in the stem bark than in the leaves. Cardiac glycosides have long been recognized for their therapeutic importance in cardiovascular disorders, alongside additional antimicrobial and anticancer activities. Likewise, saponins exhibit diverse biological properties, including membrane permeabilizing, immunomodulatory, antimicrobial, anti-inflammatory, and antiparasitic effects (Afuwape *et al*., 2025). The significantly higher saponin concentration detected in the methanol stem bark extract strengthens its pharmacological profile and may account for its enhanced anthelmintic activity observed later in this study.

5.1. Phytotoxins (anti-nutritional factors) composition of *Daniellia oliveri* leaf and stem bark extracts

Although medicinal plants are valuable sources of therapeutic phytochemicals, they may contain anti-nutritional factors (phytotoxins) that influence nutrient bioavailability and, at elevated concentrations, pose toxicological concerns. Higher concentrations of phytate in the n-hexane extracts of both the stem bark and leaves compared to their corresponding methanol extracts suggest that solvent selection considerably influences extraction efficiency, reflecting differential interactions within the plant matrix. Phytic acid is recognized as an anti-nutritional factor due to its strong chelating ability, forming insoluble complexes with essential dietary minerals like calcium, iron, zinc, and magnesium, thereby reducing their intestinal absorption. Nevertheless, moderate concentrations of phytate exhibit antioxidant activity through metal ion chelation (Afuwape *et al*., 2025). Therefore, while elevated phytate levels in n-hexane extracts may limit mineral availability if consumed excessively, they may also contribute to beneficial biological effects within acceptable limits.

The significantly lower cyanide content recorded in the leaf extracts, particularly the n-hexane leaf extract, suggests that these preparations may possess a comparatively wider safety margin. The

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levels detected require further toxicological evaluation to determine whether they remain within acceptable safety thresholds, though appropriate processing methods including boiling, solvent extraction, or drying could reduce cyanide content prior to therapeutic utilization.

Oxalates bind calcium to form insoluble calcium oxalate crystals, reducing calcium bioavailability and potentially contributing to kidney stone formation or metabolic complications when consumed excessively over prolonged periods (Gboshe, 2020). The relatively low oxalate content observed in the stem bark extracts represents an important nutritional advantage and enhances their suitability for medicinal applications compared with the leaf extracts.

The absence of a significant difference in nitrate content between the two n-hexane extracts suggests that n-hexane may preferentially extract nitrate-associated constituents from both plant parts. Although dietary nitrates are not inherently toxic and can exert beneficial health effects through nitric oxide production, excessive intake may increase the risk of methemoglobinemia and facilitate the formation of potentially carcinogenic N-nitroso compounds (Olafadehan *et al*., 2020). Consequently, monitoring nitrate concentrations remains important.

Despite the presence of phytate, cyanide, oxalate, and nitrate, the concentrations observed do not diminish the medicinal potential of the plant but highlight the need for appropriate extraction procedures, dosage optimization, and safety evaluation. The lower oxalate content and favorable phytochemical composition of the stem bark support its potential as the preferred part for pharmaceutical development, provided processing methods minimize anti-nutritional factors while preserving bioactive constituents.

5.3. Antioxidant activity of *Daniellia oliveri* leaf and stem bark extracts

As presented in Table 3a, vitamin C exhibited the highest radical scavenging activity and the lowest IC_{50} value, confirming its superior potency. Among the plant extracts, the methanol stem bark extract demonstrated the greatest antioxidant activity, evidenced by its low IC_{50} value. The inverse relationship between IC_{50} values and antioxidant activity indicates that lower values correspond to stronger free radical scavenging capacity, as less extract is required to neutralize 50% of DPPH radicals. Accordingly, the superior performance of the methanol stem bark extract suggests it contained greater quantities of antioxidant phytochemicals, consistent with the phytochemical profile where it yielded the highest concentrations of total phenolics, flavonoids, tannins, and saponins. Phenolic compounds and flavonoids are effective antioxidants because their hydroxyl groups readily donate electrons or hydrogen atoms to stabilize reactive oxygen species and terminate free radical chain reactions (Brewer, 2011; Omada and Arogba 2019; Afuwape *et al*., 2025). Their high abundance in the methanol stem bark extract provides a plausible explanation for its superior DPPH scavenging capacity. Conversely, the poor antioxidant performance of the n-hexane leaf extract is attributable to its lower concentrations of these constituents, emphasizing the importance of solvent polarity in extraction.

The DPPH assay demonstrates that *Daniellia oliveri*, particularly the methanol stem bark extract, possesses appreciable antioxidant potential. These findings support the ethnomedicinal use of the

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plant in conditions associated with oxidative stress and suggest that the stem bark represents the most promising source of natural antioxidants (Adeleye *et al*., 2014).

In Table 3b, the Ferric Reducing Antioxidant Power (FRAP) assay, which measures the ability of antioxidants to reduce ferric (Fe^{3+}) ions to their ferrous (Fe^{2+}) form through electron donation, provides complementary mechanistic information. The strong FRAP activity observed in the methanol stem bark extract is consistent with its high concentrations of electron-donating phenolic compounds and flavonoids. The close agreement between the DPPH and FRAP assays strengthens the evidence that the antioxidant activity of *Daniellia oliveri* is largely driven by its abundant phenolic constituents. Both methods consistently identified the methanol extracts as possessing greater antioxidant potential than their corresponding n-hexane extracts.

5.4. *In vitro* anthelmintic activity of *Daniellia oliveri* leaf and stem bark extracts

Anthelmintic efficacy was evaluated based on the duration required to induce paralysis in Pheretima posthuma, where shorter paralytic times reflect greater biological potency. All extracts demonstrated measurable, concentration-dependent anthelmintic activity varying by plant tissue, extraction solvent, and concentration (Table 4). The reference standard, piperazine citrate (10 mg/mL), produced paralysis at 12.50 min. Piperazine acts as a γ-aminobutyric acid (GABA) receptor agonist, inducing hyperpolarization of helminth muscle membranes to cause flaccid paralysis (Pal and Pandab, 2010).

Among the plant extracts, the methanol leaf extract exhibited the most potent anthelmintic response across all tested concentrations. At 50 mg/mL, it induced paralysis within 15.55 min, which did not differ significantly ($p > 0.05$) from its performance at 20 mg/mL (17.00 min), suggesting that maximal response under these experimental conditions was approached at 20 mg/mL. Conversely, the methanol stem bark extract exhibited longer paralytic times (37.26 min at 10 mg/mL to 31.72 min at 50 mg/mL), though increasing its concentration from 10 to 50 mg/mL significantly reduced paralysis time ($p < 0.05$), demonstrating a concentration-dependent response. The n-hexane extracts similarly confirmed leaf superiority, with the n-hexane leaf extract (50 mg/mL) achieving paralysis at 19.00 min compared to 21.10 min for the n-hexane stem bark extract at the same concentration.

The superior potency of the leaf tissue despite lower overall phenolic and tannin concentrations relative to the stem bark strongly correlates with its distinct phytochemical profile, particularly its higher alkaloid content ($p < 0.05$). While literature suggests that alkaloids, tannins, and saponins can disrupt parasite neuromuscular function, protein integrity, and membrane permeability (Maisale *et al*., 2010; Lopez-Aban *et al*., 2025), these crude extract observations represent phenotypic correlations rather than proven single-compound mechanisms. Because pure bioactive entities were not isolated in this study, exact molecular targets (specific ion-channel blockade or oxidative phosphorylation uncoupling) remain unverified and warrant future bio-guided fractionation.

5.5. Antiemetic activity of *Daniellia oliveri* leaf and stem bark extracts

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The antiemetic activity was evaluated using the copper sulfate-induced emesis model in young chicks. Copper sulfate induces emesis primarily through peripheral stimulation of the gastrointestinal mucosa, leading to the release of serotonin (5-hydroxytryptamine) from enterochromaffin cells. Released serotonin activates vagal afferent nerves transmitting impulses to the medullary vomiting center, triggering retching and vomiting (Babic and Browning, 2014); a reduction in retching frequency indicates effective antiemetic activity.

As shown in Table 5, the reference drug, metoclopramide, produced the greatest inhibition of retching (150 mg/kg produced 68.94 % reduction in retching) compared to control group. Among the plant extracts (150 mg/kg), the n-hexane stem bark extract exhibited the highest antiemetic effect (60.25 % inhibition), followed by the methanol stem bark extract (52.32 % inhibition). In contrast, the leaf extracts demonstrated weak activity, yielding 10.71 % (methanol leaf) and 14.29 % (n-hexane leaf) inhibition. Under the conditions of this screening model, the antiemetic activity of the n-hexane stem bark extract showed no statistically significant difference ($p > 0.05$) compared to metoclopramide. However, given the screening sample size ($n = 4$ per group), this outcome reflects statistical non-significance in a preliminary trial rather than established clinical or pharmaceutical equivalence.

The pronounced activity of stem bark extracts correlates with their significantly higher total phenolic and flavonoid contents ($p < 0.05$). Polyphenols such as flavonoids and other phenolic compounds are known to possess localized anti-inflammatory and antioxidant properties that may attenuate peripheral mucosal irritation or modulate local neurotransmitter dynamics (Ahmed e*t al*., 2011Zia-Ul-Haq *et al*., 2013). While metoclopramide acts primarily via central dopamine D_2 and peripheral serotonin 5-HT3 receptor antagonism (Bulbul *et al*., 2013), any proposed receptor-mediated mechanism for *Daniellia oliveri* remains speculative until active constituents are isolated and receptor-binding assays are conducted.

6. Conclusion

This study provides preliminary *in vitro* and animal model evidence supporting the comparative pharmacological potential and ethnomedicinal relevance of *Daniellia oliveri*. The findings demonstrate clear part- and solvent-dependent variations in biological efficacy; while the stem bark represents a rich source of phenolic compounds exhibiting superior antioxidant capacity and notable antiemetic activity in the chick model, the leaf extract, particularly its methanol fraction demonstrates dominant anthelmintic potency against *Pheretima posthuma*. Although anti-nutritional factors were detected, their concentrations suggest that the plant material maintains an acceptable safety margin when processed appropriately. However, because pure active compounds were not isolated, and detailed pharmacokinetic, toxicological, and molecular receptor-mechanistic investigations were beyond the scope of this preliminary study, these results should not be interpreted as definitive proof for direct clinical or pharmaceutical application. Instead, this work provides a scientific baseline that justifies further bio-guided fractionation, compound isolation, and mechanistic studies to identify the specific bioactive constituents responsible for the observed activities.

Conflict of Interest

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Authors declared no conflict of interest.

Data Availability

All supporting data and findings regarding this study can be gotten through the corresponding author upon reasonable request.

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Conceptualization, methodology and writing original draft preparation, Ibrahim G.E. and Prof. Omale J.; funding acquisition Ibrahim G.E.;, supervision, Prof. Omale J.; writing review and editing, Visualization and Validation, Omada A.A.; project administration, Olajide J.E.; Software and Resources, Omada A.A, Obajemu, B.S., Odiba, J.C., Emeje, T.O. and Adams, O.S.

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