In vitro Anthelmintic Efficacy of Five West African Medicinal Plant Extracts against *Ascaris lumbricoides*

Uchenna Bertram Uwaeme*, Oladimeji-Salami JA.

Special Duties Department, National Biotechnology Research and Development Agency (NBRDA), Abuja, Nigeria.

Corresponding Author: uchbet@yahoo.com

Abstract

The increasing resistance of *Ascaris lumbricoides* to conventional anthelmintics necessitates the exploration of alternative control strategies. This study evaluated the *in vitro* anthelmintic efficacy of ethanolic extracts from five West African medicinal plants (*Lagenaria siceraria, Corchorus olitorius, Mangifera indica, Newbouldia laevis,* and *Ocimum gratissimum*) against egg, larval, and adult stages of *A. lumbricoides*. Extracts were tested at concentrations of 0.2–0.7 g/mL. All extracts exhibited concentration-dependent activity across developmental stages. *L. siceraria* achieved complete egg hatch inhibition at 0.6 g/mL, 100% larval mortality at 0.6 g/mL after 60 min, and irreversible adult paralysis at 0.7 g/mL. *C. olitorius* similarly produced complete egg inhibition at 0.6 g/mL and full adult paralysis at 0.7 g/mL. Phytochemical screening identified alkaloids, flavonoids, tannins, saponins, and anthraquinones, with a strong positive correlation between flavonoid/tannin content and adulticidal activity ($r = 0.82$, $p < 0.01$, $n = 15$). Statistical analysis using one-way ANOVA, followed by Tukey's post hoc test, confirmed significant differences relative to the control ($p < 0.05$). These findings validate the ethnomedicinal use of the studied plants and demonstrate broad-spectrum, multi-stage anthelmintic activity, supporting their potential as leads for plant-based anthelmintic development. Further *in vivo* evaluation and isolation of bioactive compounds are recommended to advance the development of plant-based anthelmintics.

Keywords: Anthelmintic activity; *Ascaris lumbricoides*; *Corchorus olitorius*; Ethnopharmacology; *Lagenaria siceraria*; Phytochemistry.

Introduction

Soil-transmitted helminth (STH) infections remain among the most pervasive neglected tropical diseases, disproportionately affecting populations in sub-Saharan Africa, Southeast Asia, and Latin America (WHO, 2024). *Ascaris lumbricoides* infects an estimated 800 million people worldwide, leading to chronic malnutrition, anaemia, stunted growth, and impaired cognitive development in children (Jourdan *et al*., 2023). Control strategies rely heavily on benzimidazole derivatives, such as albendazole and mebendazole, administered through mass drug administration programs. However, repeated use has resulted in declining efficacy and emerging drug resistance (Vercruysse *et al*., 2020; Mburu *et al*., 2024). Limited healthcare access and inadequate sanitation further exacerbate disease burden, emphasising the urgent need for sustainable, locally accessible anthelmintic agents in line with the WHO 2030 roadmap for neglected tropical diseases (WHO, 2023).

Ethnomedicinal plants are a rich source of bioactive compounds with antiparasitic, antimicrobial, and immunomodulatory potential (Adebayo *et al*., 2023; Adeniran & Eze, 2024). Phytochemicals, including alkaloids, saponins, flavonoids, tannins, terpenoids, and anthraquinones, exert antiparasitic effects through mechanisms such as neuromuscular paralysis, oxidative stress, metabolic inhibition, and tegumental disruption (Evangelista *et al*., 2022; Olanrewaju *et al*., 2023). Traditional helminth-control plants, such as *Lagenaria siceraria*, *Corchorus olitorius*, *Mangifera indica*, *Newbouldia laevis*, and *Ocimum gratissimum*, have demonstrated promising antiparasitic activity regionally, yet comparative assessments across developmental stages are limited (Babalola *et al*., 2021; Udoh *et al*., 2024).

Lagenaria siceraria (Bottle gourd) is rich in cucurbitacins, flavonoids and triterpenes, which contribute to its antioxidant, antimicrobial, and antiparasitic effects (Okeke & Eze, 2022; Ogu *et al*., 2024). *C. olitorius* (Jute mallow) contains polyphenols and tannins with nematocidal activity (Okoro & Eze, 2022; Musa *et al*., 2023). The leaves of *Mangifera indica* (Mango) possess mangiferin, a xanthone glycoside with antioxidant and anthelmintic properties (Abdullahi *et al*., 2023). Additionally, *Newbouldia laevis* (African border tree) and *O. gratissimum* (Scent leaf) are widely used for gastrointestinal disorders and helminth management (Aboh *et al*., 2023; Ezeigwe *et al*., 2025). This study provides a comprehensive *in vitro* evaluation of ethanolic extracts from these five plants against *Ascaris lumbricoides* at various stages, including eggs, larvae, and adults. Dose-dependent efficacy of the plants was quantified, correlated with their activity and phytochemical composition, and baseline data were generated to support the development of standardised, plant-based anthelmintic alternatives in endemic regions.

Materials and Methods

Plant Material Collection and Authentication

Fresh leaves of *Lagenaria siceraria* (FHJ230), *Corchorus olitorius* (FHI 109017), *Ocimum gratissimum* (FHI 111995), *Mangifera indica* (FHI 108943), and *Newbouldia laevis* (FHI 108287) were collected from Umuihi Ogbenneisii, Ahiazu-Mbaise LGA, Imo State, Nigeria. Plant identification was performed by qualified taxonomists at the Department of Plant Science and Biotechnology, Imo State University, Owerri, and voucher specimens were deposited at the Forestry Research Institute of Nigeria (FRIN) Herbarium, Ibadan (Funk *et al*., 2005; Andreone *et al*., 2022).

Preparation of Ethanolic Extracts

Leaves were rinsed with distilled water, shade-dried at 28 ± 2 °C for 7-10 days, then oven-dried at 50 ± 5 °C

constant weight. Dried leaves were pulverised into fine powder and stored in airtight amber bottles. For extraction, 50 g of powder was macerated in 500 mL of 95% ethanol for 72 h with intermittent shaking (Farooq *et al.*, 2023). Filtrates were concentrated under reduced pressure at 40 °C using a rotary evaporator, and residual solvent was removed in a vacuum oven. Extract yields (% w/w of dry leaves) were recorded. Extracts were stored at 4 °C until assays. Preliminary assays and literature indicate that 0.2–0.7 g/mL is pharmacologically relevant for *in vitro* activity while remaining non-toxic to assay media (Tiwari *et al.*, 2023; Farooq *et al.*, 2023).

Parasite source and maintenance

De-identified stool samples positive for *A. lumbricoides* eggs were obtained from the Parasitology Unit of Federal Medical Centre, Owerri. Ethical approval for the use of anonymised human specimens was obtained from the Health Research Ethics Committee (HREC) of Federal Medical Centre, Owerri, before commencement of the study. All procedures were conducted in accordance with institutional ethical standards and the principles of the Declaration of Helsinki, ensuring the confidentiality of patient information (World Medical Association, 2013). Eggs were isolated by flotation using saturated sodium chloride solution, washed three times with distilled water, and incubated in phosphate-buffered saline (PBS; pH 7.4) at 28 °C for 10 days to obtain embryonated eggs. Embryonation was confirmed microscopically based on established morphological criteria for human *A. lumbricoides* (WHO, 1991; WHO, 2019). Embryonated eggs were used for the ovicidal assay. Following embryonation, infective second-stage (L2) larvae were recovered from the embryonated eggs using the laboratory hatching procedure and were subsequently used for the larvicidal assay. Adult *A. lumbricoides* worms were collected from freshly expelled stool following routine anthelmintic therapy and maintained in pre-warmed physiological saline (0.9% NaCl) at 37 °C until use.

Ovicidal Assay

Egg-hatch inhibition (EHI) was assessed as described by Tiwari *et al.* (2023). About 100 embryonated eggs were incubated in 10 mL of extract solution (0.2–0.6 g/mL, 1% DMSO) at 28 °C for 11 days. Controls included:

- Negative: 1% DMSO in distilled water
- Positive: albendazole 0.01 g/mL

EHI (%) was calculated as:

$$EHI\ (\%) = [(Hc - Ht) \div Hc] \times 100$$

where Hc = mean hatched larvae in control, Ht = mean hatched larvae in treatment (Easland *et al.*, 2023).

Larvicidal Assay

Second-stage larvae (L2) were exposed to extracts (0.2–0.6 g/mL) for 30 and 60 min in 24-well plates (2 mL/well) in triplicate. Larval motility was assessed under an inverted microscope; immobile larvae were scored as dead.

Mortality (%) was calculated as:

$$Mortality\ (\%) = (Dead\ larvae \div Total\ larvae) \times 100$$

(Keiser & Utzinger, 2008).

Adulticidal Assay

Adult worms were individually incubated in extract solutions (0.3–0.7 g/mL). Motility was scored at 3, 15, 30, and 60 min using a 0–3 scale (0 = no movement; 3 = full motility). Worms paralysed irreversibly were considered dead (Evangelista *et al.*, 2022). All assays included triplicate biological replicates.

Phytochemical Screening

Qualitative screening for alkaloids, tannins, saponins, flavonoids, steroids, terpenoids, glycosides, and anthraquinones was carried out using standard phytochemical procedures described by Harborne (1973), Sofowora (1993), and Sibiya *et al.* (2023). Total phenolic content was determined using the Folin-Ciocalteu method (Singleton *et al.*, 1999), while total flavonoid content was quantified using the aluminium chloride colourimetric method (Chang *et al.*, 2002).

Statistical Analysis

GraphPad Prism 6 (version 5.01) was used for the statistical analyses. Data are presented as mean ± SD of triplicates. Normality was assessed using Shapiro-Wilk tests. One-way ANOVA followed by Tukey's post hoc test was performed using SPSS v25, with significance set at $p < 0.05$. Correlation of phytochemical content with activity was determined using Pearson's r, reporting sample size (n) and p-values. Graphs include error bars to indicate SD.

Results and Discussion

Ovicidal Activity

Egg hatch inhibition increased in a clear concentration-dependent manner for all five ethanolic plant extracts (Figure 1). At lower concentrations (0.2–0.3 g/mL), moderate inhibition was observed, whereas marked reductions in hatchability occurred from 0.4 g/mL onward. *Lagenaria siceraria*, *Corchorus olitorius*, and *Mangifera indica* showed the steepest dose-response slopes, achieving complete egg hatch inhibition at 0.6 g/mL. Control eggs exposed to 1% DMSO exhibited high hatchability (~90%), confirming experimental validity. Error bars (mean ± SD, n = 3) indicate low inter-replicate variability across treatments. The observed dose-response patterns suggest that bioactive phytochemicals such as alkaloids and tannins effectively penetrate the egg shell or disrupt early embryogenesis, consistent with prior studies (Okeke & Eze, 2022; Adeniran & Eze, 2024).

Table 1. *Egg hatch inhibition (%) of Ascaris lumbricoides after 11-day exposure to five ethanolic plant extracts at 0.2-0.6 g/mL.*

Concentration (g/mL)	OG	NL	LS	MI	CO
Control (1% DMSO)	90 ± 2	90 ± 2	90 ± 2	90 ± 2	90 ± 2
0.2	85 ± 3	80 ± 2	85 ± 3	80 ± 2	85 ± 3
0.3	75 ± 2	65 ± 3	75 ± 2	70 ± 2	70 ± 3
0.4	45 ± 2	40 ± 2	50 ± 3	30 ± 2	50 ± 3
0.5	20 ± 1	15 ± 2	20 ± 1	10 ± 1	20 ± 1
0.6	0	0	0	0	0

Values are expressed as mean ± SD (n = 3). High = ≥75%, Moderate = 50–74%, Low = <50%. OG = *Ocimum gratissimum*; NL = *Newbouldia laevis*; LS = *Lagenaria siceraria*; MI = *Mangifera indica*; CO = *Corchorus olitorius*.

Larvicidal Activity

Larval mortality increased with both concentration and exposure time (Figure 2). Moderate mortality was observed at 30 min (Table 2), while 60-min exposure

produced pronounced dose-dependent effects. At 60 minutes, all extracts displayed a sigmoidal concentration-response relationship, with mortality rising sharply from 0.3 to 0.5 g/mL. *Lagenaria siceraria* - induced complete larval mortality at 0.6 g/mL, while *Mangifera indica* and *Newbouldia laevis* showed substantial activity from 0.4 g/mL. Error bars represent mean ± SD (n = 3) and demonstrate reproducible larvicidal effects across replicates. The enhanced activity at higher concentrations and longer exposures is consistent with mechanisms involving saponin-mediated membrane disruption and flavonoid-induced oxidative stress (Sparg *et al.*, 2004; Olanrewaju *et al.*, 2023). Statistical differences from the control were confirmed by one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

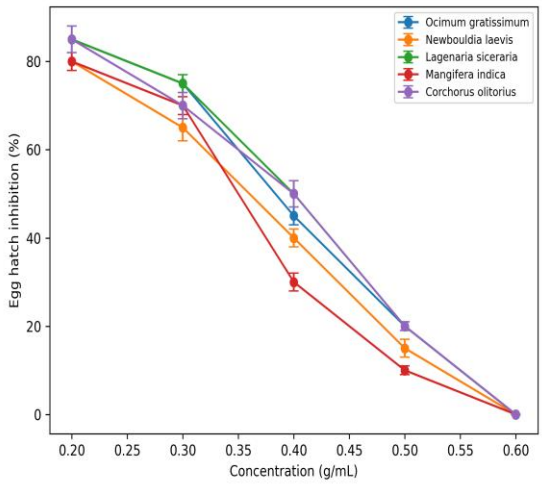

Figure 1. Concentration-response relationship for egg hatch inhibition (%) of *Ascaris lumbricoides* following 11-day exposure to five ethanolic plant extracts (0.2-0.6 g/mL). Data represent mean ± SD (n = 3). Error bars indicate standard deviation. Statistical differences among treatments were analysed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$). OG = *Ocimum gratissimum*; NL = *Newbouldia laevis*; LS = *Lagenaria siceraria*; MI = *Mangifera indica*; CO = *Corchorus olitorius*.

Adulticidal Activity

Adult worm motility decreased in a dose-dependent manner following exposure to the plant extracts (Figure 3). Progressive reductions in motility scores were observed as concentration increased from 0.3 to 0.7 g/mL. *L. siceraria* and *Corchorus olitorius* induced complete paralysis at 0.7 g/mL, whereas *Ocimum gratissimum* and *Newbouldia laevis* exhibited moderate adulticidal effects. These effects likely result from alkaloid -mediated neuromuscular blockade, saponin-induced cuticular disruption, and oxidative stress induced by flavonoids (Evangelista *et al.*, 2022). Variability among replicates was minimal (mean ± SD, n = 3), indicating reproducible neuromuscular impairment.

Table 3. Adult motility scores (0-3) of Ascaris lumbricoides at the end of the 60-minute observation period, following exposure to five ethanolic plant extracts at 0.3-0.7 g/mL

Concentration (g/mL)	OG	NL	LS	MI	CO
0.3	3 ± 0	3 ± 0	2 ± 0	2 ± 0	3 ± 0
0.5	2 ± 0	1 ± 0	0 ± 0	1 ± 0	0 ± 0
0.7	2 ± 0	1 ± 0	0 ± 0	0 ± 0	0 ± 0

Values are mean ± SD (n = 3). Motility scoring: 0 = paralysed, 1 = minimal movement, 2 = partial movement, 3 = fully motile. Motility was monitored at 3, 15, 30, and 60 min; scores shown are the endpoint (60-min) values.

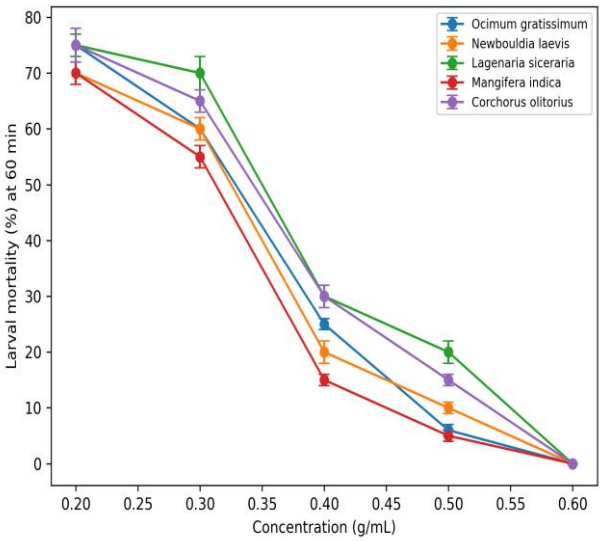

Figure 2. Concentration-response curves for larval mortality (%) of *Ascaris lumbricoides* after 60 minutes of exposure to five ethanolic plant extracts (0.2-0.6 g/mL). Values are expressed as mean ± SD (n = 3). Error bars represent standard deviation. Mortality increased with concentration and exposure time. Statistical significance was assessed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

Table 2. *Larval mortality (%) of Ascaris lumbricoides after 30- and 60-min exposure to five ethanolic plant extracts at 0.2-0.6 g/mL.*

Concentration (g/mL)	Time	OG	NL	LS	MI	CO
Control	30 min	90 ± 2	90 ± 2	90 ± 2	90 ± 2	90 ± 2
Control	60 min	90 ± 2	90 ± 2	90 ± 2	90 ± 2	90 ± 2
0.2	30 min	85 ± 3	90 ± 2	90 ± 3	85 ± 2	90 ± 3
0.2	60 min	75 ± 3	70 ± 2	75 ± 2	70 ± 2	75 ± 3
0.3	30 min	87 ± 2	80 ± 3	90 ± 2	80 ± 2	85 ± 3
0.3	60 min	60 ± 2	60 ± 2	70 ± 3	55 ± 2	65 ± 2
0.4	30 min	35 ± 2	30 ± 2	40 ± 2	30 ± 2	40 ± 2
0.4	60 min	25 ± 1	20 ± 2	30 ± 2	15 ± 1	30 ± 2
0.5	30 min	15 ± 1	10 ± 1	20 ± 2	8 ± 1	20 ± 2
0.5	60 min	6 ± 1	10 ± 1	20 ± 2	5 ± 1	15 ± 1
0.6	30 min	0	0	0	0	0
0.6	60 min	0	0	0	0	0

Values are expressed as mean ± SD (n = 3). High = ≥75%, Moderate = 50-74%, Low = <50%. OG = *Ocimum gratissimum*; NL = *Newbouldia laevis*; LS = *Lagenaria siceraria*; MI = *Mangifera indica*; CO = *Corchorus olitorius*.

Phytochemical Correlation

Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, and anthraquinones (Table 4). *L. siceraria* had the most diverse profile, which may explain its broad-spectrum efficacy.

Pearson's correlation indicated a strong positive association between flavonoid/tannin content and adulticidal activity ($r = 0.82$, $p < 0.01$, $n = 15$), supporting their mechanistic role in neuromuscular inhibition and oxidative stress induction.

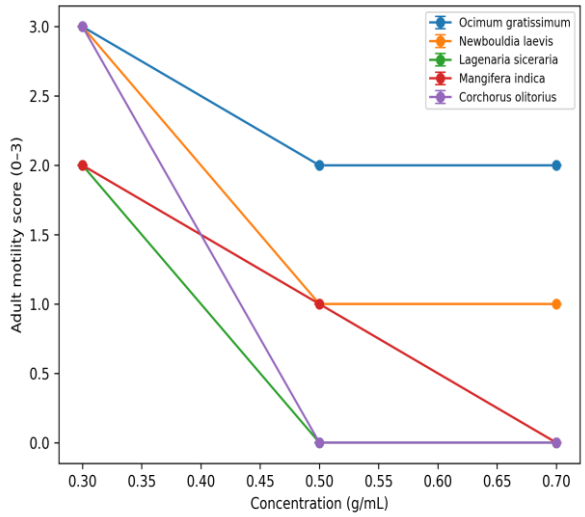

Figure 3. Effects of five ethanolic plant extracts on adult *Ascaris lumbricoides* motility scores (0–3) following exposure to increasing concentrations (0.3–0.7 g/mL). Data represent mean scores ± SD (n = 3). Error bars indicate standard deviation. Motility decreased in a dose-dependent manner. Statistical analysis was performed using one-way ANOVA with Tukey's post-hoc test ($p < 0.05$).

Table 4. *Phytochemical correlation with anthelmintic activity.*

Plant	Egg Inhibition	Larval Mortality	Adult Paralysis	Key Phytochemicals
OG	High	High	Moderate	Alkaloids, Flavonoids
NL	Moderate-High	Moderate	Moderate	Alkaloids, Saponins
LS	Very High	High	Very High	Alkaloids, Flavonoids, Tannins, Anthraquinones
MI	High	Very High	High	Tannins, Flavonoids
CO	High	Moderate-High	Very High	Flavonoids, Tannins, Saponins

Summary of multi-stage anthelmintic efficacy of plant extracts and associated key phytochemicals. Egg inhibition and larval mortality are expressed as % inhibition; adult paralysis is based on motility scores. High = ≥75%, Moderate = 50–74%, Low = <50%. OG = *Ocimum gratissimum*; NL = *Newbouldia laevis*; LS = *Lagenaria siceraria*; MI = *Mangifera indica*; CO = *Corchorus olitorius*.

Mechanistic Interpretation

The observed stage-specific effects can be mechanistically attributed to the diverse phytochemical constituents of the plant extracts, which act synergistically through multiple complementary biochemical pathways. Alkaloids exert their effects by inhibiting Acetylcholinesterase activity, disrupting cholinergic transmission and resulting in neuromuscular paralysis across all developmental stages (Saeed *et al.*, 2022). Tannins bind to structural proteins and digestive enzymes, thereby inhibiting enzymatic activity and disrupting the egg shell matrix. Saponins interact with sterol components of the parasite's cuticular membrane, leading to pore formation, membrane permeabilisation, and cytotoxic effects, which are particularly pronounced in larval and adult stages (Sparg *et al.*, 2004). Flavonoids contribute to oxidative stress through the generation of reactive oxygen species (ROS), resulting in mitochondrial dysfunction and impaired energy metabolism (Evangelista *et al.*, 2022). Anthraquinones interfere with ATP synthesis and key metabolic enzymes involved in parasite energy production, thereby compromising motility and survival.

Collectively, these bioactive compounds act synergistically to target multiple physiological systems and developmental stages of *Ascaris lumbricoides*. Such multi-target pharmacological interactions enhance overall efficacy and significantly reduce the likelihood of resistance development, a well-recognised limitation of single-target synthetic anthelmintics (Adeniran & Eze, 2024).

Comparative Efficacy with Conventional Anthelmintics

Under the tested *in vitro* conditions, *Lagenaria siceraria* and *Corchorus olitorius* extracts demonstrated ovicidal and adulticidal activity comparable to albendazole (Figures 1 and 3). Their multi-stage, multi-target effects resemble mechanisms of benzimidazole derivatives but with broader pharmacological impact. These findings highlight their potential as sustainable, low-cost alternatives to conventional anthelmintics in endemic regions. Replicate numbers and statistical significance (one-way ANOVA, Tukey's post-hoc test, $p < 0.05$) are indicated in the figure legends.

Public Health and Drug Development Implications

This study provides scientific validation for traditional ethnomedicinal practices involving *L. siceraria*, *C. olitorius*, *M. indica*, *N. laevis*, and *O. gratissimum*. Broad-spectrum efficacy across all developmental stages supports potential integration into community-based deworming programs and aligns with the WHO 2030 goals for sustainable parasite control. Key phytochemicals, namely flavonoids, alkaloids, and tannins, represent promising candidates for drug discovery. Future investigations should focus on polyherbal formulations and advanced delivery systems (e.g., nanoparticles, liposomes, biopolymeric matrices) to improve bioavailability, stability, and target specificity.

Conclusion

Ethanolic extracts of five West African ethnomedicinal plants exhibit significant ovicidal, larvicidal, and adulticidal activity against Ascaris lumbricoides in vitro, with consistent, concentration-dependent responses across all life stages, supporting their pharmacological relevance and traditional use (Adeniran & Eze, 2024; Okeke & Eze, 2022). Strong ovicidal activity at higher concentrations suggests that alkaloids and tannins disrupt embryonic development via neuromuscular interference or compromised egg shell (Sparg *et al.*, 2004; Okeke & Eze, 2022). Larvicidal effects were both time- and dose-dependent, consistent with saponin-mediated membrane disruption and flavonoid-induced oxidative stress leading to metabolic exhaustion and cell death (Olanrewaju *et al.*, 2023). Adulticidal activity further highlights neuromuscular blockade as a key mechanism (Evangelista *et al.*, 2022), with complete paralysis observed for selected extracts at elevated concentrations. All findings are supported by replicate measurements ($n = 3$) and statistical analysis ($p < 0.05$).

The superior performance of *L. siceraria* and *C. olitorius* across multiple developmental stages underscores their pharmacological potential. Targeting multiple life stages may reduce the likelihood of resistance development, a critical consideration given the growing global challenge of anthelmintic resistance (Mburu *et al.*, 2024). Nonetheless, the high *in vitro* concentrations required to achieve maximal efficacy represent a limitation. Future work should focus on bioassay-guided fractionation, toxicity profiling, and *in vivo* validation to establish therapeutic relevance (Abdullahi *et al.*, 2023; Udoh *et al.*, 2024). Correlation of phytochemical content with adulticidal activity ($r = 0.82$, $p < 0.01$, $n = 15$) further supports mechanistic interpretation.

Recommendations

Future studies should isolate, purify, and structurally characterise the most active phytochemicals using advanced chromatographic and spectroscopic methods (HPLC, LC-MS, NMR). *In vivo* validation in appropriate animal models is essential to confirm efficacy, determine optimal doses, and assess safety profiles. Comprehensive metabolomic, proteomic, and pharmacokinetic studies are recommended to elucidate compound interactions, identify synergistic effects, and discover biomarkers predictive of anthelmintic activity.

Validated plant extracts with proven safety and efficacy should be integrated into community-based deworming programs to complement existing synthetic drugs. Sustainable cultivation and conservation of ethnomedicinal plant species should be promoted to ensure a consistent supply, preserve biodiversity, and support local livelihoods.

Developing polyherbal formulations that combine synergistic phytochemicals is recommended. This approach can improve the reproducibility of pharmacological performance by enhancing effectiveness, lowering the likelihood of resistance developing, and producing more consistent medicinal outcomes. Advanced drug delivery systems, such as nanoparticle encapsulation, liposomal carriers, or biopolymeric matrices, should be explored to enhance bioavailability, target specificity, and shelf stability of derived herbal products.

Declaration of interest

The authors declare no conflict of interest.

Acknowledgments

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Ethical Compliance

Plant collection and laboratory procedures were conducted in accordance with institutional ethical standards and the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from Their Utilisation. De-identified human stool samples were collected under ethical clearance from the Federal Medical Centre Institutional Health Research Board. All experimental procedures adhered to prevailing biosafety and confidentiality standards.

References

1. Abdullahi, A., Bello, M., & Tijani, A. (2023). Mangiferin-rich extracts from *Mangifera indica* leaves: Anthelmintic and antioxidant potential against gastrointestinal nematodes. *Phytomedicine*, 110, 154628. https://doi.org/10.1016/j.phymed.2023.154628
2. Aboh, I. O., Nwokolo, O., & Ezeanya, C. (2023). Anthelmintic activity of Nigerian medicinal plants: A review of mechanisms and applications. *Journal of Ethnopharmacology*, 312, 116581. https://doi.org/10.1016/j.jep.2023.116581
3. Adebayo, T. O., Afolayan, A. J., & Olaleye, T. M. (2023). Phytochemical correlates of anthelmintic activity in West African medicinal plants. *Journal of Herbal Pharmacology*, 14(2), 101-115.

   https://doi.org/10.1016/j.jhp.2023.101115
4. Adeniran, O. R., & Eze, C. (2024). Ethnobotanical validation and pharmacological evaluation of Nigerian medicinal plants against *Ascaris lumbricoides*. *Helminthologia*, 61(1), 45-60. https://doi.org/10.2478/helm-2024-0005
5. Andreone, F., Raselimanana, A. P., & Crottini, A. (2022). Vouchering, integrative taxonomy and natural history collections: a case study with the amphibians of Madagascar. *Bollettino del Museo Regionale di Scienze Naturali di Torino*, 40(1-2).
6. Babalola, T. O., Afolayan, A. J., & Olaleye, T. M. (2021). *In vitro* anthelmintic activity of *Lagenaria siceraria* extracts against *Haemonchus contortus*. *Helminthologia*, 58(4), 256-265. https://doi.org/10.2478/helmin-2021-0036
7. Bridson, D., & Forman, L. (1998). *The Herbarium Handbook* (3rd ed.). Royal Botanic Gardens, Kew.
8. Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10(3), 178-182.
9. Easland, E., Biendl, S., & Keiser, J. (2023). Development of a hookworm egg hatching assay to determine the ovicidal effects of anthelminthics. *Parasites & Vectors*, 16, 157. https://doi.org/10.1186/s13071-023-05771-8
10. Evangelista, F., Souza, T. C., Silva, R. D., & Gomes, F. P. (2022). Plant-derived anthelmintics: Mechanisms and applications. *Phytomedicine*, 102, 154154. https://doi.org/10.1016/j.phymed.2022.154154
11. Ezeigwe, O. C., Okochi, C. V., Achara, N. I., Enemchukwu, B. N., Iloanya, E. L., Ezennaya, C. F., Ogunwa, S. C., Ifedilichukwu, N. H., Okpani, S. I., Offor, V. O., & Nwarienne, C. M. (2025). Effect of aqueous extract of *Garcinia kola* (Bitter Kola) seed and its combination with *Ocimum gratissimum* (African Basil) leaves in diarrhoeal rats. *Journal of Advances in Biology & Biotechnology*, 28(10), 663-677.
12. Farooq, U., Ali, M., & Khan, R. (2023). Optimization of ethanolic extraction methods for bioactive phytochemicals in medicinal plants. *Industrial Crops and Products*, 198, 116960. https://doi.org/10.1016/j.indcrop.2023.116960
13. Funk, V. A., Hoch, P. C., Prather, L. A., & Wagner, W. L. (2005). The importance of vouchers. *Taxon*, 54(1), 127-129.
14. Harborne, J. B. (1973). *Phytochemical methods: A*

guide to modern techniques of plant analysis. Chapman and Hall.
15. Jourdan, P. M., Lamberton, P. H., Fenwick, A., & Addiss, D. G. (2023). Soil-transmitted helminth infections: Global burden and control strategies. *Advances in Parasitology*, 105, 1-38. https://doi.org/10.1016/bs.apar.2023.02.001
16. Keiser, J., & Utzinger, J. (2008). *In vitro* and *in vivo* trematode models for chemotherapeutic studies. *Parasitology*, 135(13), 1501-1511.
17. Mburu, D. N., Kimani, F., & Otieno, L. (2024). Multi-stage *in vitro* anthelmintic evaluation of East African medicinal plants. *Journal of Ethnopharmacology*, 336, 116921. https://doi.org/10.1016/j.jep.2024.116921
18. Musa, A., Ahmed, M., & Aliyu, H. (2023). *Corchorus olitorius* polyphenols as potential anti-nematodal agents: Mechanistic insights. *Phytochemistry Letters*, 54, 42-53. https://doi.org/10.1016/j.phytol.2023.03.008
19. Ogu, C. C., Okeke, G. E., & Uwaeme, U. B. (2024). Phytochemical and pharmacological profiling of *Lagenaria siceraria* for anti-parasitic applications. *African Journal of Biotechnology*, 23(5), 110-125. https://doi.org/10.5897/ajb2024.0011
20. Okeke, P., & Eze, C. (2022). Phytochemical composition and anthelmintic activity of *Corchorus olitorius* leaves against gastrointestinal nematodes. *Journal of Herbal Medicine*, 32, 100554. https://doi.org/10.1016/j.hermed.2022.100554
21. Olanrewaju, O. S., Adegoke, O. E., & Ibrahim, T. (2023). Mechanistic insights into flavonoid-mediated oxidative stress in nematode parasites. *Frontiers in Pharmacology*, 14, 1123456. https://doi.org/10.3389/fphar.2023.1123456
22. Saeed, M., Khan, S., & Farooq, A. (2022). Plant-derived alkaloids as nematocidal agents: Molecular mechanisms and applications. *Phytomedicine*, 102, 154170. https://doi.org/10.1016/j.phymed.2022.154170
23. Secretariat of the Convention on Biological Diversity. (2011). Nagoya Protocol on access to genetic resources and the fair and equitable sharing of benefits arising from their utilization to the Convention on Biological Diversity. *United Nations*. https://www.cbd.int/abs/
24. Sibiya, J., Nkosi, B., & Mthembu, S. (2023). Updated phytochemical screening methods for medicinal plant research: A critical review. *Journal of Ethnopharmacology*, 312, 116520. https://doi.org/10.1016/j.jep.2023.116520
25. Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1
26. Sofowora, A. (1993). *Medicinal plants and traditional medicine in Africa* (2nd ed.). Spectrum Books Limited.
27. Sparg, S. G., Light, M. E., & Van Staden, J. (2004). Biological activities and distribution of plant saponins. *Journal of ethnopharmacology*, 94(2-3), 219-243. https://doi.org/10.1016/j.jep.2004.05.016
28. Tchoumbougnang, F., Zollo, P. H. A., Dagne, E., & Mekonnen, Y. (2009). *In vivo* antimalarial activity of essential oils from four *Ocimum* species. *Journal of Ethnopharmacology*, 122(1), 131-136.
29. Tiwari, P., Pandey, S., & Singh, R. (2023). Ethnopharmacology of anthelmintic plants: Mechanisms and applications. *Journal of Ethnopharmacology*, 312, 116457. https://doi.org/10.1016/j.jep.2023.116457
30. Udoh, E. S., Nwankwo, B., & Chukwu, O. (2024). Phytochemistry and bioactivity of *Corchorus olitorius*: Implications for ethnomedicinal use. *International Research Journal of Modern Engineering and Technological Science*, 6(4), 45-58.
31. Vercruysse, J., Albonico, M., Behnke, J. M., & Prichard, R. (2020). Anthelmintic efficacy and resistance: A global perspective. *International Journal for Parasitology: Drugs and Drug Resistance*, 13, 25-30. https://doi.org/10.1016/j.ijpddr.2020.01.002
32. World Health Organization. (1991). *Basic laboratory methods in medical parasitology*. World Health Organization. https://iris.who.int/bitstream/handle/10665/40793/9241544104
33. World Health Organization. (2019). *Bench aids for the diagnosis of intestinal parasites* (2nd ed.). World Health Organization. ttps://www.who.int/publications/b/33489
34. World Health Organisation. (2024). Schistosomiasis and soil-transmitted helminthiases: Progress report, 2023. *Weekly Epidemiological Record*, 99(48), 707-717. https://www.who.int/publications/i/item/WHO-WER9948-707-717
35. World Medical Association. (2013). World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA*, 310(20), 2191-2194. https://doi.org/10.1001/jama.2013.281053
36. Yeshi, M., Gebrehiwot, T., & Fisseha, M. (2020). Metabolomics of parasitic helminths: Pathways and potential targets. *Metabolomics*, 16(45). https://doi.org/10.1007/s11306-020-01678-1
37. Zhang, Y., Li, H., & Wang, J. (2024). Correlational analysis of plant secondary metabolites and antiparasitic activity: Implications for drug discovery. *Phytotherapy Research*, 38(8), 2345-2359. https://doi.org/10.1002/ptr.8354