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Phytochemical Composition and In Vitro Anthelmintic Activity of Vitex doniana Leaf Extract against Taenia solium and Taenia saginata

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ABSTRACT

Helminth infections are still a public health and veterinary problem and continue to be of interest for medicinal plants as a source of bioactive compounds against helminths. In this study, the qualitative phytochemical composition of the leaf extracts of Vitex doniana and the in vitro anthelmintic activity of the methanolic extract of the leaves of Vitex doniana were evaluated. The fresh leaves of V. doniana were harvested, identified, shade dried, ground and extracted separately with methanol, ethanol and distilled water by maceration method. The qualitative phytochemical screening was carried out for alkaloids, flavonoids, tannins, saponins, phenols, glycosides, steroids and terpenoids. The methanolic extract was chosen for anthelmintic activity due to its relatively high phytochemical content and tested at concentrations of 25, 50, 100 and 200 mg/mL. Paralysis and death times were recorded, and albendazole (20 mg/mL) was used as a non-equivalent positive control. The methanolic extract was rich in flavonoids (+++) and saponins (+++) and the ethanolic extract was rich in phenols (+++). The anthelmintic assay showed that the paralysis and death times were reduced in a concentration-dependent manner. The extract caused paralysis and death times of 27.8 ± 1.2 and 60.4 ± 1.8 min, respectively, against specimens reported as T. solium and 30.7 ± 1.3 and 64.2 ± 1.9 min, respectively, against specimens reported as T. saginata at 200 mg/mL. Albendazole (20 mg/mL) produced shorter paralysis and death times; however, because the highest extract concentration (200 mg/mL) was 10-fold higher than that of the positive control, this comparison is non-equivalent and is reported descriptively only. A concentration-matched positive control would be required for any valid potency comparison. The results obtained in this study indicate that the extract of V. doniana leaves has measurable concentration-dependent in vitro anthelmintic activity, and further research is warranted to explore the bioactive components of the plant. The results, however, do not prove clinical efficacy or the compounds responsible for the activity. Taxonomic confirmation of test organisms, quantitative phytochemical profiling, bioassay-guided fractionation, toxicity studies, mechanistic investigations and suitably designed in vivo studies are recommended.

KEYWORDS: Vitex doniana; phytochemical screening; anthelmintic activity; Taenia; medicinal plants; methanolic extract.

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Introduction

Plants have been used for alleviating human suffering from the very beginning of human civilization, and records of the use of plants are available from about 5000 years ago (Hassan & Abdulkaim, 2023; Sen & Samanta, 2014). After a long period, human beings learned to classify plant materials to suit their needs in

meeting their basic life activities. One of these basic activities is the application of plants and plant extracts for the cure of ailments because of their healing properties (Nazim, 2012). It has been reported that plants and herbs are important sources of drugs for herbal and modern medicine (Ayo et al., 2013). The healing properties of these herbs largely depend on the active principles found in the herbal extracts called phytochemicals (Ayo et al., 2013). In the Nigerian context, medicinal plants continue to play a central role in primary healthcare, and their antimicrobial and antiparasitic properties have been the subject of increasing scientific attention (Ugboko et al., 2020).

Helminth infections comprise a diverse group of parasitic diseases that disproportionately affect populations in settings where sanitation, veterinary control, and access to diagnosis and treatment are limited. Although soil-transmitted helminths constitute a major global group, foodborne cestode infections also remain important because transmission is closely linked to food preparation, sanitation, and animal husbandry. The World Health Organization recognizes taeniasis caused by *Taenia solium*, *T. saginata*, and *T. asiatica* as a foodborne parasitic infection; among these, *T. solium* is of public-health importance because humans may develop cysticercosis, including neurocysticercosis, after ingestion of eggs (WHO, 2022, 2025).

Taeniasis caused by adult *Taenia* tapeworms may be mild or asymptomatic, but *T. solium* has an additional and clinically important tissue-invasive stage. Control therefore depends not only on treatment of infected people but also on interruption of transmission through appropriate sanitation, food safety, diagnosis, and animal-health measures. The continued need for effective antiparasitic interventions has stimulated research into compounds from natural sources. Importantly, the existence of anthelmintic drug resistance in some helminth systems and the limitations associated with available treatments provide a rationale for discovering new chemical leads, but in vitro plant activity should not be interpreted as evidence of clinical effectiveness.

Medicinal plants are important sources of secondary metabolites which are chemically diverse such as phenolics, flavonoids, alkaloids, tannins, terpenoids and saponins. *Vitex doniana* Sweet (Lamiaceae), black plum, is a medium sized deciduous tree with a rounded crown. Leaves palmately 3–7 (mostly 5) leaflets, lower leaflets distinctly smaller, mostly hairless, except for a few stellate hairs on midrib below, dark green above, paler below, apex rounded or notched, sometimes abruptly acuminate. Flowers in branched axillary heads, blue, violet or white with lower lobe darker. Fruit is ellipsoid, c. 3 × 2 cm, fleshy, hairless, purple-black, edible. The characteristic foliage of *V. doniana* is shown in Plate 1.



Taxonomy of *Vitex doniana* Sweet (Lamiaceae)	
Kingdom:	Plantae
Phylum:	Tracheophyta
Class:	Magnoliopsida
Order:	Lamiales
Family:	Lamiaceae
Genus:	*Vitex*
Species:	*Vitex doniana*
Author:	Sweet
Common names:	Black plum (English); Dinya (Hausa); Orin (Yoruba); Eru (Igbo)

Plate 1: Leafs of *Vitex doniana* Sweet (Lamiaceae)

Plate 1. Leaves of *Vitex doniana* Sweet (Lamiaceae), showing the characteristic foliage and taxonomic classification of the species.

A wide range of phytochemical constituents have been reported in *V. doniana*, and recent reviews outline its reported antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and other biological activities (Irampagarikiy et al., 2025; Mahmoud Dogara et al., 2025; Das et al., 2022). Experimental studies have also shown phytochemical constituents of *V. doniana* leaves and biological activities of leaf extracts, which warrants further investigation of the species as a source of bioactive molecules (Ushie et al., 2022; Agbafor & Nwachukwu, 2011). Phytochemical studies of *V. doniana* have revealed the presence of flavonoids,

saponins, alkaloids, phenolic compounds, terpenes and other metabolite classes, while recent analytical studies have employed chromatographic techniques to identify metabolites in the leaves of *V. doniana* (Irampagarikiy et al., 2025).

However, the anthelmintic activity of *V. doniana* leaves is still relatively under-explored, especially regarding concentration-response relationships against *Taenia* specimens. The present study was thus designed to determine the qualitative phytochemical composition of the methanol, ethanol and aqueous leaf extracts of *V. doniana* and to assess the in vitro anthelmintic activity of the crude leaf extract at graded concentrations. The working hypothesis was that the leaf extract would have measurable, concentration-dependent effects on the paralysis and death times under the experimental conditions employed.

Materials and Methods

Study design

The study comprised two components: (i) qualitative phytochemical screening of *V. doniana* leaf extracts prepared using methanol, ethanol, and water; and (ii) an in vitro anthelmintic assay using graded concentrations of the crude extract. All experiments were conducted in triplicate unless otherwise stated.

Collection and authentication of plant material

Leaves of *V. doniana* were collected from uncultivated farmlands around Fago Town, Sandamu Local Government Area, Katsina State, Nigeria. The plant was identified and authenticated at the herbarium unit, Department of Biology, Federal University Dutsinma, by an expert, and the voucher number of the plant collected was 2572.

Sample preparation

The plant leaves were separated from the root, washed, air-dried, and pulverized using a grinding machine. The pulverized material was kept in a polyethylene bag and refrigerated until use.

Extraction of pulverized leaves by maceration

The pulverized leaf sample (200 g) was extracted separately with 500 cm^3 of methanol, ethanol, and distilled water in 1000 cm^3 conical flasks. The flasks were tightly corked to reduce solvent evaporation and contamination, and the mixtures were left to stand for seven days with intermittent shaking. Each mixture was then filtered to remove plant residue from the filtrate. The methanolic and ethanolic filtrates were concentrated under reduced pressure at 40 °C by rotary evaporator and the aqueous filtrate was concentrated by appropriate low temperature evaporation method. The crude extracts obtained were left to cool, weighed and kept in the refrigerator until they were needed for further analysis. Percentage yields were calculated on a dry-weight basis of the pulverized leaf material, using the formula: percentage yield = (weight of dried extract / weight of dry pulverized leaf material) × 100.

Qualitative phytochemical screening

Qualitative phytochemical tests were conducted following the method of Edeoga et al. (2005). Alkaloids, flavonoids, tannins, saponins, phenols, glycosides, steroids and terpenoids were investigated. The intensity categories reported were + (present), ++ (moderately present), and +++ (highly present), and − (absent). Screening was done once for each extract.

Glycosides. One part (0.5 g) of the extract was dissolved in 2.5 M H_2SO_4 (2.5 cm^3), boiled, cooled and neutralized with 20% KOH. Then 5 cm^3 of Fehling's solutions A and B (1:1) was added to the neutralized mixture and boiled. The brick-red precipitate formed showed the presence of glycosides (Sofowora, 1993).

Detection of saponins. A small amount of the sample was diluted with distilled water to 20 cm^3 and shaken for 15 minutes, and the foam/frothing that remained stable for some minutes was a positive sign of the presence of saponins (frothing test) (Sofowora, 1993; Hassen et al., 2024).

Detection of tannins. Two drops of iron (III) chloride reagent were added to 5 cm^3 of the extract. The bluish-black or green precipitate formed was an indication of the presence of tannins ($FeCl_3$ test) (Evans, 2002).

Detection of flavonoids. A solution of 10% NaOH solution was added to a small amount of the extract. Heavy cream precipitate was formed, which meant that flavonoids were present (Hassen et al., 2024).

Detection of alkaloids. 0.005 g of the extract was added to a small amount of dilute HCl, stirred and filtered. The filtrate was then tested separately with Mayer's, Wagner's and Dragendorff's reagent by the methods followed by Hassen et al. (2024). A few cm^3 of the filtrate was added to a portion (1 cm^3) of Mayer's reagent.

The presence of alkaloids was indicated by the development of creamy or white colour. A few cm^3 of filtrate was added to a portion (1 cm^3) of Wagner's reagent. Reddish-brown coloration was an indication of the presence of alkaloids. A few cm^3 of the filtrate was added to a portion (1 cm^3) of Dragendorff's reagent. A reddish-brown coloration developed, which meant that alkaloids were available.

Detection of steroids. A small amount of extract was dissolved in a small amount of chloroform (2 cm^3) and 2 cm^3 of concentrated H_2SO_4 was added. Formation of a red coloration indicated the availability of steroids (Dhania et al., 2023).

Collection, maintenance, and identification of helminth specimens

Worm collection. The anthelmintic assay was performed on specimens reported as *Taenia saginata* and *T. solium*. All worms were of approximately equal size (5 cm). They were collected from Katsina abattoir and washed in Petri dishes containing 0.9% neutral phosphate-buffered saline (PBS) maintained at 37 ± 1 °C in a glass-panelled microbiological incubator. The worms were identified based on gross morphological features (scolex and proglottid arrangement) by a parasitologist at the Department of Biology, Federal University Dutsinma. No molecular confirmation was performed; therefore, the species-level identity of the specimens is reported as "reported as" throughout this manuscript. Abattoir permission was obtained prior to collection, and no ethical approval was required because the specimens were obtained from routine slaughterhouse material.

Preparation of test solutions

The crude extract was dissolved in distilled water to obtain concentrations of 25, 50, 100, and 200 mg/mL. Albendazole at 20 mg/mL served as the positive control, while distilled water served as the negative control. Because the highest extract concentration (200 mg/mL) was 10-fold higher than that of the positive control, the albendazole comparison is non-equivalent and is reported descriptively only.

Selection of methanolic extract for anthelmintic assay

Based on the preliminary phytochemical screening, the methanolic extract showed a comparatively higher diversity and/or abundance of detected phytoconstituents than the ethanol and aqueous extracts. Since several of the phytochemical groups detected, particularly flavonoids, saponins, and phenolic compounds, have been associated with potential anthelmintic activity, the methanolic extract was selected for further in vitro anthelmintic evaluation. The selection was therefore based on the phytochemical profile of the extracts, with the assumption that the greater representation of potentially bioactive constituents in the methanolic extract could provide a broader basis for evaluating the anthelmintic potential of *Vitex doniana* leaves.

In vitro anthelmintic assay

Anthelmintic activity was evaluated according to the method of Ghosh et al. (2005), with modifications. Three worms of the same type were placed in Petri dishes containing the test preparations. The test solutions were prepared freshly before the experiment. Paralysis was recorded when no movement was observed except after vigorous shaking. Death was recorded after confirming absence of movement following vigorous shaking and subsequent exposure to warm water (50 °C), as reported in the original procedure. Each concentration was tested in three independent replicates (n = 3), with three worms per replicate. Worms in the negative control were observed for up to 180 min; if no paralysis or death occurred within this period, the result was recorded as "no effect."

Results

Results of the weight extracted from methanol, ethanol, and water fractions of *V. doniana* leaf extracts and their respective percentage yields are presented in Table 1.

Table 1. Weight extracted from methanol, ethanol, and water fractions and their respective percentage yields

Solvent	Weight of extract (g)	Percentage yield (%)
Methanol	24.0	12.0
Ethanol	19.0	9.5
Water	30.0	15.0

Percentage yield was calculated on a dry-weight basis of the pulverized leaf material (200 g) used for each extraction. Values are means of triplicate extractions.

As observed from Table 1, the water-soluble components had the highest extraction yield of 15.0% (30.0 g),

followed by methanol with 12.0% (24.0 g), and the ethanol fraction with the least, 9.5% (19.0 g). The percentage yield was highest for the most polar solvent (water) and lowest for ethanol under the conditions used.

Phytochemical screening

The qualitative phytochemical profile of *V. doniana* leaf extracts is summarized in Table 2. The reported anthelmintic results are presented in Tables 3 and 4. Because the original dataset contains summary values only, the present revision does not add inferential significance markers.

Table 2. Qualitative phytochemical screening of *V. doniana* leaf extracts

Phytochemical	Methanol	Ethanol	Aqueous
Alkaloids	++	++	+
Flavonoids	+++	++	++
Tannins	++	++	+
Saponins	+++	++	++
Phenols	++	+++	++
Glycosides	+	+	+
Steroids	+	+	−
Terpenoids	++	++	+

Scale: + = present; ++ = moderately present; +++ = highly present; − = absent.

Table 3. In vitro anthelmintic activity of *V. doniana* methanolic leaf extract against specimens reported as *T. solium*

All values represent Mean ± SD; n = 3 in each group. Species-level identity was based on gross morphology only and was not confirmed molecularly. The albendazole comparison is non-equivalent because the highest extract concentration (200 mg/mL) was 10-fold higher than that of the positive control (20 mg/mL); no direct potency comparison is made.

Table 4. In vitro anthelmintic activity of *V. doniana* methanolic leaf extract against specimens reported as *T. saginata*

Treatment	Concentration (mg/mL)	Paralysis time (min)	Death time (min)
Negative control	0	No effect	No effect
V. doniana leaf extract	25	80.5 ± 2.3	142.8 ± 3.4
V. doniana leaf extract	50	62.4 ± 2.1	115.7 ± 2.9
V. doniana leaf extract	100	44.9 ± 1.7	87.6 ± 2.0
V. doniana leaf extract	200	27.8 ± 1.2	60.4 ± 1.8
Non-equivalent reference control (albendazole)	20	18.2 ± 0.8	39.6 ± 1.1

All values represent Mean ± SD; n = 3 in each group. Species-level identity was based on gross morphology only and was not confirmed molecularly.

Concentration-response patterns

The reported means show a consistent descriptive concentration-response pattern. For specimens reported as *T. solium*, paralysis time decreased from 80.5 min at 25 mg/mL to 27.8 min at 200 mg/mL, while death

time decreased from 142.8 to 60.4 min. For specimens reported as *T. saginata*, paralysis time decreased from 84.2 to 30.7 min and death time from 149.3 to 64.2 min across the same concentration range. These patterns indicate increasing activity with increasing crude-extract concentration under the conditions of the assay. One-way ANOVA with Tukey's post-hoc test was not performed because the original dataset contained summary values only; the present results should therefore be interpreted descriptively, and the concentration-response patterns reported here do not by themselves establish statistical significance.

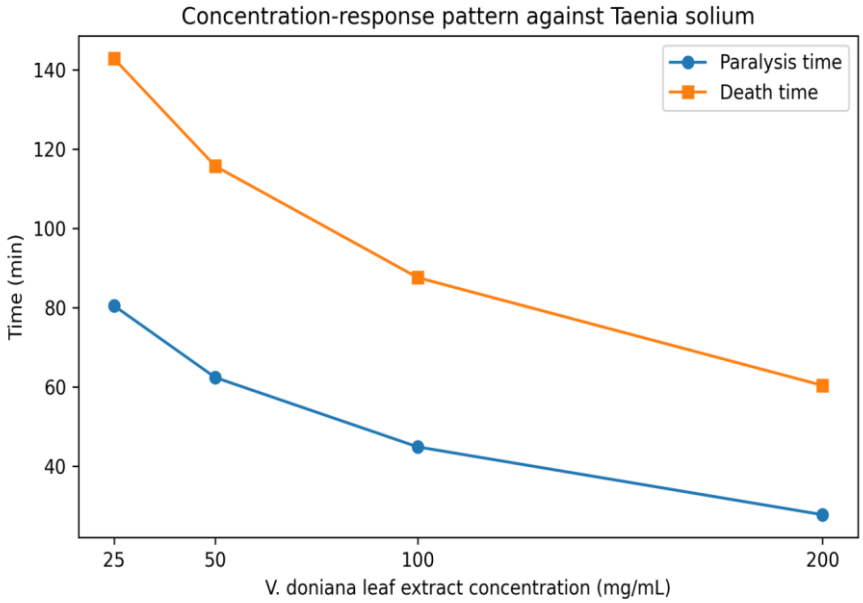


Figure 1. Reported mean paralysis and death times across *V. doniana* leaf extract concentrations for specimens reported as *T. solium*.

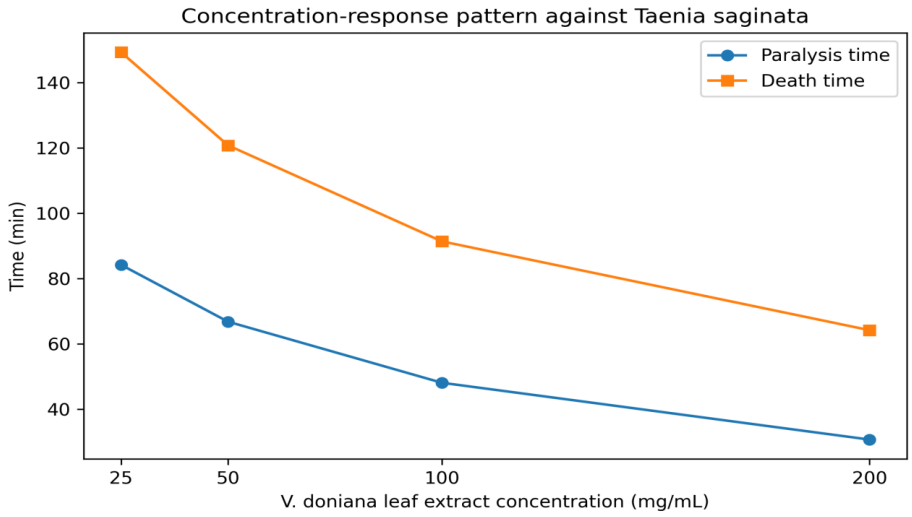


Figure 2. Reported mean paralysis and death times across *V. doniana* leaf extract concentrations for specimens reported as *T. saginata*.

Discussion

The present study provides two principal observations: first, *V. doniana* leaves contain multiple classes of secondary metabolites; and second, the tested methanolic extract produced shorter paralysis and death times as concentration increased. The phytochemical pattern is broadly consistent with recent literature describing *V. doniana* as a chemically diverse medicinal plant containing flavonoids, saponins, alkaloids,

phenolic compounds, terpenoids, steroids, and related metabolites (Irampagarikiy et al., 2025; Mahmoud Dogara et al., 2025; Das et al., 2022; Ushie et al., 2022).

The selection of the methanolic extract for the anthelmintic assay was guided by its comparatively rich phytochemical profile. The methanolic extract showed high (+++) levels of flavonoids and saponins, while these constituents were moderately detected (++) in the ethanolic and aqueous extracts. Phenols were highly detected (+++) in the ethanolic extract, indicating that the distribution of phytoconstituents varied according to the extraction solvent. The relatively strong representation of flavonoids and saponins in the methanolic extract provided a rational basis for its selection for the subsequent anthelmintic assay, as these classes of secondary metabolites have been associated with potential antiparasitic and anthelmintic effects.

The observed anthelmintic activity may be associated, at least in part, with the combined effects of the phytochemical constituents present in the extract. The experiments were conducted in three independent replicates, and the results are expressed as mean ± standard deviation (SD). These observations are descriptive only, and no statistical inference is drawn. However, the qualitative phytochemical screening does not establish a direct causal relationship between any specific phytochemical group and the observed anthelmintic activity. Further quantitative phytochemical analysis, fractionation and isolation of active constituents, and mechanistic studies are therefore necessary to identify the compounds responsible for the observed effects.

Albendazole produced shorter paralysis and death times than the plant extract at the tested concentrations. However, this comparison is non-equivalent: albendazole was tested at 20 mg/mL, whereas the highest extract concentration was 200 mg/mL, a 10-fold difference. The apparent superiority of albendazole under these conditions therefore cannot be interpreted as a direct potency comparison. The difference may reflect the greater potency of the standardized drug, differences in concentration, bioavailability in the assay medium, or the fact that the plant material is a chemically complex crude extract. A concentration-matched comparison would be required to draw any conclusion about relative potency, and no such conclusion is made here.

Because the specimens were identified only by gross morphology, the species-level attribution of the observed activity should be regarded as provisional. Molecular confirmation of the test organisms is required before species-level conclusions can be drawn.

The findings are also consistent with the broader literature on *V. doniana*. Recent reviews have documented extensive traditional use and a diverse phytochemical and pharmacological literature for the species (Irampagarikiy et al., 2025; Mahmoud Dogara et al., 2025). Recent analytical work has further demonstrated that *V. doniana* leaf extracts can contain identifiable metabolites detectable by GC-MS (Ushie et al., 2022).

Strengths and limitations

Strengths

- The study examines a locally available medicinal plant of documented ethnobotanical interest.
- Multiple extraction media were investigated for qualitative phytochemical screening.
- The anthelmintic assay included graded extract concentrations and a conventional positive control.
- Both paralysis and death times were measured after three independent experimental replicates, and the results were expressed as mean ± SD.

Limitations

- The phytochemical analysis is qualitative/semi-quantitative and does not identify individual compounds or their concentrations.
- The taxonomic identity of the test organisms was based on gross morphology only and was not confirmed molecularly; species-level conclusions should therefore be treated with caution.
- The positive-control comparison is non-equivalent because albendazole was tested at a 10-fold lower concentration than the highest extract concentration.
- No statistical analysis was performed because only summary values were available; the results are therefore presented as descriptive preliminary observations only.
- No toxicity, selectivity, mechanistic, or in vivo data were generated; therefore, clinical or therapeutic efficacy cannot be inferred.

Conclusion

V. doniana leaf extracts contained several classes of phytochemicals, with the qualitative profile varying according to extraction solvent. The tested crude leaf extract also showed a descriptive concentration-dependent reduction in paralysis and death times against the helminth specimens evaluated, whereas albendazole produced shorter times under the reported assay conditions; however, this comparison is non-equivalent because the positive control was tested at a 10-fold lower concentration than the highest extract concentration, and no potency comparison is therefore made. These findings are preliminary and descriptive and do not establish statistical significance or therapeutic efficacy. They support further investigation of *V. doniana* as a potential source of anthelmintic bioactive compounds, but they do not establish therapeutic efficacy or identify the active constituents. Species-level conclusions concerning *T. solium* and *T. saginata* should be made only after the taxonomic identity and developmental stage of the test organisms have been adequately verified, ideally by molecular methods.

Recommendations and future research

- Quantify total phenolic, flavonoid, and other relevant phytochemical contents and, where resources permit, perform HPLC, LC-MS, or GC-MS profiling.
- Use bioassay-guided fractionation to determine whether activity is associated with particular fractions or compounds.
- Investigate mechanism of action and establish concentration-response parameters such as EC_{50}/IC_{50} where experimentally appropriate.
- Conduct validated toxicity and selectivity studies before considering any translational or therapeutic implications.
- Perform molecular identification of *Taenia* specimens in future studies to confirm species-level identity.
- Include concentration-matched positive controls in future assays to enable valid potency comparisons.
- Include raw replicate-level data in future reports to permit inferential statistical analysis.

Declarations

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

Authors' Contributions

Datin, M. U. conceived and designed the study, carried out the field collection of plant material, performed the extraction and phytochemical screening, conducted the in vitro anthelmintic assay, analysed and interpreted the data, and drafted the manuscript.

Ismail Abdulkarim contributed to the study design, supervised the phytochemical screening and anthelmintic assay, assisted with data interpretation, and critically revised the manuscript for important intellectual content.

Both authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable. No human or animal subjects were used in this study. Plant material was collected and authenticated in accordance with institutional guidelines, and the voucher specimen (number 2572) was deposited at the herbarium unit, Department of Biology, Federal University Dutsinma. Helminth specimens were obtained from routine slaughterhouse material at Katsina abattoir with prior permission from the abattoir management; no ethical approval was required because the specimens were not obtained from living human or animal subjects.

Consent for Publication

Not applicable. This manuscript does not contain any individual person's data in any form.

Availability of Data and Materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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Disclaimer

The findings reported in this manuscript are preliminary and descriptive in nature. No clinical or therapeutic efficacy is claimed. The limitations of the study, including the non-equivalent positive control comparison, the morphological-only identification of test organisms, and the absence of inferential statistical analysis, have been transparently disclosed in the manuscript.

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