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Original Article

In Vitro anthelminthic activity of the aqueous leaf extract of *Cassia Occidentalis* against *Ascaris Lumbricoides*

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Abstract

Intestinal nematodes affect children in impoverished populations. Conventional anthelminthic drugs have been frequently utilized to manage these infections. However, limitations such as cost, toxic effects, and resistance have shifted towards herbal medicines in low-to middle-income countries. There is a lack of evidence supporting the effectiveness of Cassia occidentalis plant leaf extract in managing helminth infections in Uganda. This study, therefore, sought to determine the phytochemical composition of Cassia occidentalis aqueous leaf extract, its acute toxicity profile, and its in-vitro anthelmintic activity against Ascaris lumbricoides. Leaves of Cassia occidentalis were collected from Bushenyi-Ishaka Municipality, dried to obtain a powder, and macerated with distilled water to create an aqueous extract. This extract underwent phytochemical screening and acute toxicity testing following established guidelines. The anthelmintic activity was evaluated using an adult worm mortality assay. The study found that the aqueous leaf extract of Cassia occidentalis had significant anthelmintic activity at a concentration of 40 mg/ml ($p<0.041$). Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, and glycosides, but no steroids were found. The extract had an LD_{50} of over 5000 mg/kg body weight, as it caused no mortality. The study concluded that Cassia occidentalis has significant anthelmintic activity and a high LD_{50}, supporting its traditional use in managing helminth infections.

Keywords: In-Vitro; anthelminthic; aqueous; leaf extract; *cassia occidentalis; ascaris lumbricoides*

Introduction

Helminth refers to a wide range of parasitic worms that inhabit the body (WHO, 2020). Helminth infections are a threat to both human beings and livestock alike, especially in developing countries, where they can lead to malnutrition, anemia, and pneumonia with significant economic and social consequences [1]. Most infections are limited to tropical and subtropical regions with inadequate sanitation and poor water quality. In particular, they can also occur in travelers who have visited areas with high endemicity, and some may also be found in temperate environments. Nematodes hold the title of being the most prevalent multicellular organisms across the globe, boasting an estimated number of about 100,000 to 1,000,000 species. Approximately 25,000 species of nematodes have been identified, over half of which are parasitic, affecting humans, animals, or plants [2].

Human parasitic nematodes are generally classified into two major types: intestinal nematodes, which primarily inhabit the intestinal lumen, and tissue nematodes, which reside in body tissues during their adult stage. Intestinal parasitic nematodes, the most prevalent and enduring type of human parasitic worms, affect around two billion individuals globally, predominantly in developing nations [3]. The World Health Organization further estimates that by 2025, approximately 57% of people in developing countries will be infected by helminths if control strategies are not implemented and enforced [2]. These intestinal parasitic nematodes mainly include *Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale, Necator americanus, Strongyloides stercoralis*, *Enterobius vermicularis* and *Capillaria philippinensis* [2,3].

Infections from these parasites can negatively impact human growth, nutrition, cognitive development, academic performance, work efficiency, and pregnancy outcomes, potentially leading to a significant decline in quality of life [4]. Most intestinal parasitic infections are found in children, where they also indirectly contribute to a substantial disease burden by weakening the immune system. This leads to heightened vulnerability to diseases such as malaria, HIV/AIDS, and tuberculosis [4].

Control of these parasites in livestock and humans depends on contemporary anthelmintic treatments, along with enhancements in sanitation and public health education. An ideal anthelmintic possesses a wide range of effectiveness, a high cure rate, safety for the host, and cost-efficiency [4,5]. Nevertheless, many synthetic drugs fail to fulfil these criteria. The parasites' resistance to current medications and their significant expense justifies the exploration of new anthelmintic compounds. In this context, herbal medications are seen as affordable, safe, effective, and accessible to disadvantaged groups in developing countries such as Uganda [6].

Owing to the challenges faced by modern pharmaceuticals, a large portion of the global population turns to traditional medicinal remedies. Indeed, in many tropical developing countries, the majority of people utilize medicinal plants as anthelmintic agents [4]. Plant-based traditional medicine remains a primary healthcare source for over 80% of the global population, especially in developing countries [1,7].

Cassia occidentalis is a widely distributed and frequently utilized plant in Uganda and is known locally in the Luganda and Runyankole languages as “omutanjoka” or “omwitanjoka” (‘worm killer’) and ‘Coffee senna’ in English. *Cassia occidentalis* has been traditionally utilized by rural communities in Uganda for its numerous medicinal properties, including antimicrobial, anthelmintic, insecticidal, antioxidant, anti-anxiety, antidepressant, antimutagenic, antidiabetic, wound healing, and hepatoprotective effects, anti-inflammatory, analgesic, antipyretic and other effects [5]. These have been reported to be due to the presence of many phytochemicals including alkaloids, anthocyanosides, phenolics, proteins, phlobatannins, steroids, tannins, flavonoids, anthraquinones, saponins, terpenes, resins, balsams, amino acids, carbohydrates, sugars and cardiac glycosides [5,8]. However, despite the use *of Cassia occidentalis* by local communities in the management of helminthiasis, there is scanty data published about the anthelminthic effect and safety of its aqueous leaf extracts thus the need for this study. This study therefore seeks to evaluate the in-vitro anthelmintic activity of *Cassia occidentalis* aqueous leaf extract against gastro-intestinal nematodes.

Problem statement

Intestinal nematodes infect many of the world's poorest populations, with children mainly being affected, and it constitutes a formidable public problem, with as many as 400 million children infected [2]. Over time, traditional anthelmintic medications like albendazole, mebendazole, ivermectin, piperazine, and niclosamide have been widely used to treat helminth infections in humans and livestock. However, the use and effectiveness of these drugs, especially among populations in low- to middle-income countries, have been restricted due to their cost, toxic effects, and reported resistance. Consequently, many have turned to less expensive alternatives, primarily herbal medicines. Numerous studies have recorded herbal remedies' traditional or folkloric applications for treating helminthic infections [4]. Scientific justifications for using some of these medicinal plants have been extensively documented, with *Cassia occidentalis* inclusive.

However, there is a dearth of evidence to support using the aqueous leaf extract of *Cassia occidentalis* in managing helminth infections in Uganda. More so, there is a common notion held by all people that herbal medicines are harmless since they are natural. However, this is a perilous oversimplification, as diverse effects attributed to herbal medicines have been reported [3]. This study, therefore, established the *in-vitro* anthelmintic efficacy and safety of *Cassia occidentalis* aqueous leaf extract against *Ascaris lumbricoides*.

Materials and methods

Study design

This study employed an *in-vitro* experimental study design to assess the anthelminthic activity of *Cassia occidentalis* aqueous leaf extract against *Ascaris lumbricoides*. The study assessed the qualitative phytochemical composition of *Cassia occidentalis* leaves and evaluated the activity and toxicity of its aqueous extract in laboratories at Kampala International University Western Campus.

Study site

This study was done at the Pharmacology and Toxicology laboratory, in the School of Pharmacy at Kampala International University, Western Campus. Extraction was carried out in the Pharmacology Laboratory.

Study materials

Plant identification and collection

Cassia occidentalis leaf samples were collected from Bwegiragyje in Ishaka municipality, Bushenyi district, and then taken to a plant taxonomist at the Department of Science Laboratory Technology/Biology, Mbarara University of Science and Technology, for identification and authentication. After that, the specimen was stored in the herbarium at the KIU Pharmacognosy laboratory.

After they had been identified, fresh Cassia occidentalis leaves were collected early in the morning, paying attention to avoid damaged and/or diseased leaves. They were packed and transported in sterile nylon bags to the laboratory for processing and drying.

Extraction of Cassia occidentalis leaves

The extraction of the plant product was performed using distilled water, chosen for its effectiveness as a solvent. To create the powdered plant material, we adhered to standard preparation methods. Specifically, 1000 gm of the powdered leaf extract was combined with 10000 ml of distilled water in a flask, maintaining an optimal 1:10 (w/v) ratio. This mixture was allowed to stand for 24 h in a wooden cabinet, ensuring periodic shaking to enhance extraction efficiency. Afterward, the mixture was carefully strained through cotton wool and filtered using Whatman No.1 filter paper to capture a pure filtrate. This filtrate was then dried in an oven at 35 °C, ensuring the preservation of its beneficial properties. The resulting concentrate was weighed, stored in an airtight glass bottle, and kept at 4 °C for future use. Prior to initiating the anthelmintic and oral acute toxicity evaluation experiments, we prepared a stock solution of the extract using distilled water, guaranteeing a reliable foundation for our research.

Determination of phytochemicals from Cassia occidentalis leaves

The aqueous leaf extract of Cassia occidentalis underwent qualitative phytochemical screening using previously described methods [6]. The plant extracts were then tested for the presence of saponins, tannins, alkaloids, glycosides, flavonoids, and steroids using the technique which is described below:

*Te*st for saponins

Two milliliters (2 ml) of the crude extract were mixed with 5 ml of distilled water in a test tube, shaken vigorously, and heated to boiling. The presence of stable foam indicated the existence of saponins.

Test for tannins

Two milliliters of raw extract were mixed with an equal volume of a 2% ferric chloride solution (2 ml of 2% $FeCl_3$). The appearance of blue-green or black hues indicated the presence of tannins.

Test for alkaloids

Alkaloid detection was performed using Dragendorff's test, where 1 or 2 drops of the reagent were added to 1 ml of the sample. The appearance of a distinct yellow precipitate indicated the presence of alkaloids.

Test for glycosides

Two milliliters of crude extract were combined with 2 ml of chloroform, and then 2 ml of concentrated sulfuric acid (H_2SO_4) was carefully added. Gently shaking the mixture and observing a reddish-brown ring at the interphase suggested the presence of glycosides.

Test for flavonoids

Three drops of ammonia solution were added to a test tube containing 1 ml of crude extract, followed by 0.5 ml of concentrated hydrochloric acid (HCl). The appearance of a pale-brown color indicated the presence of flavonoids.

Test for steroids

Two milliliters of crude extract were combined with 2 ml chloroform and evaporated until dry. Afterward, 2 ml of concentrated sulfuric acid (H_2SO_4) was added, and the mixture was heated for approximately 2 min. The appearance of a red color suggested the presence of steroids.

In vitro anthelmintic activity of Cassia occidentalis leaves

The *in-vitro* anthelmintic activity was conducted according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines, with minor modifications in how parasites were collected and in the mortality assays of adult worms as outlined below:

Parasite collection

Mature adult parasites of *Ascaris lumbricoides* were collected directly from the intestines of slaughtered pigs at an abattoir. The worms were moved to the pharmacology laboratory using a phosphate-buffered saline (PBS, pH 6.4). The identification and separation of adult and mature females were conducted using a microscope, adhering to the techniques outlined by Hansen and Perry [9].

Adult Worms Mortality Assay (AWMA)

The adult worms' mortality assay was carried out using the method detailed by Ngaradoum [4]. Mature, living *Ascaris lumbricoides* worms were collected directly from the intestines of pigs post-slaughter at an abattoir. The worms were maintained in PBS at pH 6.4 and transported to the Pharmacology and Toxicology laboratories at Kampala International University Western Campus. Here, five worms were placed in triplicate into individual Petri dishes for each treatment, kept at a laboratory temperature of 25-30 °C:

- Phosphate Buffer Saline (pH 6.4)
- *Cassia occidentalis* aqueous leaf extract diluted in PBS at concentrations 10, 20, and 40 mg/ml.
- Albendazole will be used as a positive control at 10, 20, and 40 mg/ml of equivalent concentrations.

The reduction in movement and/or the death of the worms subjected to the treatments served as an indicator of anthelmintic efficacy. Worm movement was monitored at various time intervals - 1, 2, 4, 8, and 24 h after exposure. During each observation, the number of mobile worms was tallied. Non-motile worms were removed from the Petri dish and exposed to lukewarm PBS for about 5 to 10 min; those that regained mobility were counted as alive, while those that did not were considered dead. The mortality rate of the worms for each concentration of plant extract was determined using the following formula below:

- Mortality (%) = (Number of dead worms/Number of worms in culture) x 100

Acute toxicity study

Laboratory animals

Albino rats weighing between 160-180 gm were used for the experiment. These were purchased from the Pharmacology and Toxicology animal house at Kampala International University Western Campus. The animals were maintained on portable water and a commercial rodent diet. All the institutional, national, and international guidelines for the care and use of laboratory animals were adhered to throughout this study [10].

Housing, feeding, and husbandry

The albino rats were housed in wooden cages at room temperature under a 12 h light-dark cycle and were allowed free access to standard rat feed and clean drinking water. Animals in this study were handled according to the National Institute of Health guidelines [11] for the care and use of laboratory animals [12].

Acute toxicity test

The acute oral toxicity test of the aqueous leaf extract from Cassia occidentalis was conducted on Albino Wistar rats using Lorke's method, as detailed by Silva et al. [13]. Twelve female nulliparous and non-pregnant Albino Wistar rats, each 6 weeks old and weighi

ng about 150-170 gm, were utilized in the study. Stratified random sampling divided the rats into various phases and dosage groups. In phase I, three groups of three animals each were established, and in phase II, three additional groups were formed, each including one animal. In phase I, animals within the same group were distinctly marked for easy identification and housed together in distinctly labeled cages. For phase II, each animal was individually placed in a specifically labeled cage.

Before receiving a single dose of the *Cassia occidentalis* aqueous leaf extract, the rats underwent an 18 h fasting period but had free access to drinking water (*ad libitum*). Additionally, they were housed in clean cages to avoid coprophagy. The solution was delivered in a single bolus dose to the rats via oral gavage, utilizing a curved and ball-tipped gastric gavage needle attached to a syringe.

Phase I:

- Group I (3 animals): the group received 100 mg/kg of the *Cassia occidentalis* aqueous leaf extract orally.
- Group II (3 animals): The group orally received 500 mg/kg of the *Cassia occidentalis* aqueous leaf extract.
- Group III (3 animals): The group orally received 1000 mg/kg of the *Cassia occidentalis* aqueous leaf extract.

The treated animals underwent a fasting period of 1 h immediately following the administration of the test substance, during which they were continuously monitored. Subsequent observations were made intermittently over the first 24 h and then frequently for the next 14 days.

Phase II:

- Group I (1 animal) received 1600 mg/kg of the *Cassia occidentalis* aqueous leaf extract orally.

- Group II (1 animal): The Animal received 3200 mg/kg of the Cassia *occidentalis* aqueous leaf extract orally.
- Group III (1 animal): The Animal received 5000 mg/kg of the *Cassia occidentalis* aqueous leaf extract orally.

In Phase II, the observation procedure implemented in Phase I was repeated. Observations included gross behavioral changes such as loss of appetite, hair erection, lacrimation, tremors, convulsions, salivation, diarrhea, mortality, and other signs of toxicity, which were noted and recorded daily.

If any of the animals die, the median lethal dose (LD_{50}) will be calculated using the geometric mean between the lowest dose that resulted in death and the highest dose that did not. If no deaths occur by the end of the experiment, the LD_{50} is considered higher than the highest dose tested. The LD_{50} is determined using the following formula;

Where, D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

Statistical data analysis

All results obtained were expressed as mean ± standard error of the mean (SEM). The statistical significance was determined using a One-way Analysis of Variance (ANOVA) followed by a Turkey or Dunnett post hoc test to compare group variations. The results were considered significant at $p \leq 0.05$. The analyzed data has been presented using tables and graphs where necessary. The analysis used Statistical Package for Social Scientists (SPSS) software version 23.

Ethical considerations

Approval to carry out the study was obtained from the School of Pharmacy and the Research Ethics Committee (KIU-REC) at Kampala International University before initiating the research. A combination of administrative controls, containment principles, laboratory practices and procedures, safety equipment, emergency preparedness, and laboratory facilities were used to help ensure that biosafety protocols are always adhered to and properly observed.

Results

Percentage yield of Cassia occidentalis aqueous leaf extract

The results show that the yield from the aqueous leaf extract was calculated as a percentage of mass yield. The extract yields are indicated in Table 1.

Table 1. Percentage yield of *Cassia occidentalis* aqueous leaf extract.

Plant part	Weight of powder (g)	Weight of concentrate (g)	Percentage yield (%)
Cassia occidentalis leaves	1000	88.5	8.85

Phytochemical screening

The preliminary phytochemical screening of *Cassia occidentalis* leaves revealed the following phytochemicals: saponins, tannins, glycosides, alkaloids, and flavonoids. However, steroids were absent in the leaves.

Table 2. Qualitative phytochemicals screening results of *Cassia occidentalis* leaves.

Phytochemical	Result
Saponins	++
Tannins	++
Flavonoids	+++
Alkaloids	+++
Glycosides	++
Steroids	-

Acute toxicity profile of Cassia occidentalis aqueous leaf extract

Acute toxicity behavioural changes

Behavioural changes observed during the first six hours of administration of the *Cassia occidentalis* aqueous leaf extract included hyperactivity, piloerection, sedation, reduced activity, and urination. Details of the behavioural changes are shown in Table 3.

Table 3. Observed behavioural alterations in the acute toxicity assessment of aqueous *Cassia occidentalis* leaf extract.

Characteristic	100mg/kg	500mg/kg	1000mg/kg	1600mg/kg	3200mg/kg	5000mg/kg
Reduced activity	+	+	+	+	-	+
Sedation	-	+	+	+	-	+
Piloerection	+	-	-	+	+	+
Urination	+	-	-	-	-	+
Hyperactivity	+	+	+	+	+	+
Death	None	None	None	None	None	None

+ observed; - not observed

Table 3 presents the outcomes following a one-time administration of the crude extracts from *Cassia occidentalis*. No rats died during the first 6 h of extract administration and after 14 days of observation. An increase in the extract dose led to an increase in adverse effects.

LD50 calculation

Where, D0 = Highest dose that gave no mortality,

D100 = Lowest dose that produced mortality.

Given that no animals died even at the highest dose tested (5000 mg/kg), it is safe to report the LD_{50} value of the aqueous *Cassia occidentalis* leaf extract as greater than 5000 mg/kg.

Anthelminthic activity of Cassia occidentalis aqueous leaf extract

Below are Figures 1 and 2 showing various concentrations of the extract and albendazole that resulted in paralysis and mortality of *Ascaris lumbricoides*.

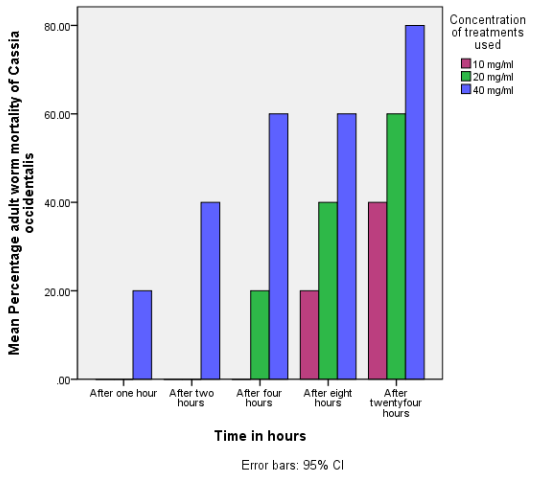

Figure 1. Mean adult worm mortality of *Ascaris lumbricoides* exposed to *Cassia occidentalis* aqueous leaf extract.

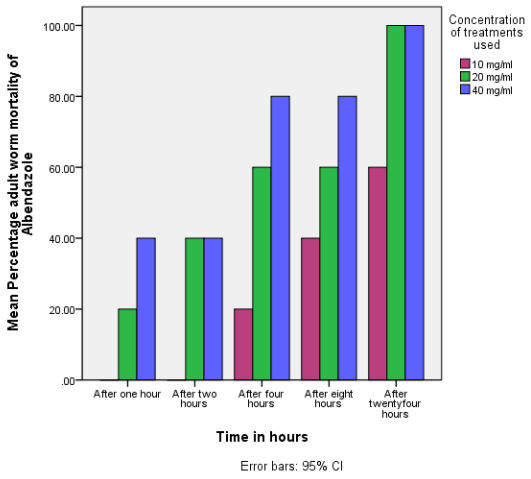

Figure 2. Mean adult worm mortality of Ascaris lumbricoides exposed to albendazole.

According to the study results in Figure 1, the mean percentage mortality of *C. occidentalis* against *A. lumbricoides* increased with time, with the highest mortality observed and the highest concentration tested (40 mg/ml). Regarding the results in Figure 2, the mean percentage mortality of albendazole against *A. lumbricoides* was much higher compared to *C. occidentalis* and was seen much earlier in all the concentrations.

Study findings in Table 4 indicate that when the percentage mortality of the two treatments used was compared against the concentrations tested, a statistically significant difference in the mean percentage mortality of the 10 mg/ml and 40 mg/ml *C. occidentalis* extract concentrations ($P = 0.041$) was

noted. However, no statistically significant difference was found in the mean percentage mortality of the albendazole concentrations studied.

Table 4. Anthelminthic effect of treatments used against *Ascaris lumbricoides*.

Treatment used		Mean± SEM	*P*-value
Percentage of adult worm mortality of *Cassia occidentalis* after 24 h	10 mg/ml	12.00 ± 8.00*	0.41
	20 mg/ml	28.00 ± 12.00	
	40 mg/ml	52.00 ± 10.19*	
Percentage of adult worm mortality of albendazole after 24 h	10 mg/ml	24.00 ± 11.66	0.085
	20 mg/ml	56.00 ± 13.27	
	40 mg/ml	68.00 ± 12.00	

* $P < 0.005$; SEM= Standard error of the mean.

Discussion

The results of this study demonstrate that the aqueous leaf extract of *Cassia occidentalis* exhibits significant anthelmintic activity against *Ascaris lumbricoides*, supporting its traditional use in the management of helminthic infections.

Phytochemical screening of Cassia occidentalis

The phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, and glycosides, which are known to contribute to the plant's medicinal properties. This finding aligns with studies by Liu et al., [3], who identified the same phytochemicals in their research. As mentioned earlier, the different phytochemical content within a plant part can be attributed to several environmental factors [14]. These secondary metabolites found in medicinal plants have been previously documented for their various biological activities, including anthelmintic, antidiarrheal, and antimicrobial effects. For instance, tannins have been reported to disrupt the energy generation in parasites by interfering with oxidative phosphorylation, leading to their death [15]. Saponins have been shown to cause damage to the parasite's cuticle, facilitating their elimination. As revealed in the phytochemical analysis, the absence of steroids in the extract suggests that the anthelmintic activity observed is not dependent on steroidal compounds, which are often associated with anti-inflammatory effects rather than direct anthelmintic action [4]. Flavonoids have also been found to be efficacious against various parasitic nematodes [1].

Preliminary phytochemical screening is crucial for identifying chemical compositions in plant materials that can be utilized subsequently in drug discovery and development. Moreover, these tests assist in quantitatively estimating and separating pharmacologically active chemical compounds.

Acute toxicity profile of Cassia occidentalis

The acute toxicity study of the crude extracts derived from *Cassia occidentalis* revealed that the extract has an LD_{50} greater than 5000 mg/kg, indicating a high safety margin. The behavioral changes observed at higher doses, such as hyperactivity and reduced activity, are consistent with the known effects of some phytochemicals on the central nervous system in the extract. These findings suggest that higher doses may induce mild neurotoxic effects while the extract is relatively safe, warranting further investigation.

The behavioral changes noted with the aqueous leaf extract of the plant at the highest dose tested (5000 mg/kg) include reduced activity, sedation, piloerection, urination, and hyperactivity. These effects can be attributed to the plant's effects on both the autonomic and central nervous systems rather than enzymatic.

Anthelminthic activity of Cassia occidentalis

The anthelmintic activity was dose-dependent, with higher concentrations of the extract resulting in increased worm mortality, which aligns with study findings from research by [16], who demonstrated that receptor saturation occurs with rising concentrations of the active component. The comparison with albendazole, a standard anthelmintic drug, showed that while the extract was less potent, it still

exhibited significant efficacy, especially at higher concentrations. This suggests that *Cassia occidentalis* could be a viable alternative, particularly in resource-limited settings where access to conventional anthelmintic drugs is restricted.

The study evaluated the anthelminthic activity of the *Cassia occidentalis* aqueous leaf extract against *Ascaris lumbricoides* using the adult worm death assay. The study noted that all concentrations of the treatments used caused some degree of mortality of the worms. Paralysis and mortality in adult worms were also observed, which could be attributed to a synergistic impact resulting from a combination of active compounds [16]. The enhanced larval and adult worm activity observed with both the *Cassia occidentalis* extract and albendazole against *Ascaris lumbricoides* may be linked to the fact that adult worms represent the feeding stages of the parasite, and this suggests that both *Cassia occidentalis* extract and albendazole might exert their effects through ingestion and penetration of the cuticle [3].

The phytochemicals present in the extract were likely to be responsible for its anthelminthic activity, and principally, tannins increase the availability of providing animals with digestible proteins through the formation of protein complexes in the rumen and impairing energy generation by interfering with oxidative phosphorylation and, reduce gastrointestinal metabolism, ultimately killing the worm [14]. Furthermore, alkaloids possess anti-oxidizing effects, which could minimize nitrate generation, benefit protein synthesis, and inhibit the transfer of sucrose from the stomach to the small intestine, thereby decreasing glucose support to the helminths—conversely, saponins cause vacuolization and disintegration of teguments [15].

Conclusion

The aqueous leaf extract of *Cassia occidentalis* has demonstrated significant in-vitro anthelmintic activity against *Ascaris lumbricoides*, supported by its rich phytochemical composition, which includes alkaloids, flavonoids, tannins, saponins, and glycosides. The extract is relatively safe with an LD_{50} greater than 5000 mg/kg, though high doses may lead to mild neurotoxic effects. These findings validate the traditional use of *Cassia occidentalis* in managing helminthic infections and suggest its potential as an alternative anthelmintic agent, especially in areas where conventional drugs are inaccessible or too costly. Further studies are recommended to explore this plant's full therapeutic potential and establish its efficacy and safety in clinical settings.

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Authors contribution

All authors were involved in data analysis, drafting the manuscript, and critically revising it. They have also agreed to take responsibility for all aspects of the work.

Declaration of interest

The authors declare no conflict of interest.

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