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Effects of Ethanolic Extracts of *Tithonia diversifolia* and *Azadirachta indica* on *Haemonchus contortus* in Goats

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ABSTRACT

Infestation of gastrointestinal worms (helminths) contributes significantly to neonatal mortality and reduced growth performance in livestock animals. The present study was conducted to determine the phytochemical composition and the *in vitro* potency of ethanolic extracts of *Tithonia diversifolia* and *Azadirachta indica* on motility inhibition on *Haemonchus contortus*. *Tithonia diversifolia* and *Azadirachta indica* were obtained from farmers in Bulambuli district of Uganda and were treated with 70% ethanol as an extraction solvent. A standard phytochemical procedure was used for qualitative analysis. The *in-vitro* experiment was conducted using 2.5 mg/ml, 5 mg/ml, 10 mg/ml, and 20 mg/ml for each plant ethanolic extract. Moreover, phosphate buffer saline (PBS) was utilized as the control. Phytochemical analysis revealed the presence of tannins, alkaloid salts, saponins, flavonoids, steroid glycosides, anthracenosides, coumarins, and anthocyanosides in ethanolic extracts. A dosage of 20 mg/l of *Tithonia diversifolia* and *Azadirachta indica* indicated motility inhibition of adult *Haemonchus contortus* after 2.55 hours and 2.1 hours, respectively, compared to the PBS control group. In conclusion, both plant extracts showed anthelmintic activity leading to the mortality of the worms. The ethanolic extracts of *Azadirachta indica* were faster in causing mortality of *Haemonchus contortus* than those of *Tithonia diversifolia* at the same dose rate of 20 mg/ml.

Keywords: *Azadirachta indica, Haemonchus contortus*, Plant extract, *Tithonia diversifolia*, Wormicide

INTRODUCTION

Helminthiasis caused by worm infestation is among the most important diseases affecting livestock productivity, leading to reduced growth rates, decreased milk production, and in severe cases, animal mortality (Karshima et al., 2018; Reman and Abidi, 2022). Yuguda et al. (2018) reported that gastrointestinal helminths are the major source of economic losses in livestock. In Europe, the annual cost of treating helminth infections is estimated at 1.8 € billion (Charlier et al., 2020). In Indonesia, Zalizar et al. (2023) reported a 20% prevalence of worm infestation in beef cattle during the dry season, with a higher prevalence observed in bulls compared to females. Similarly, in Nigeria, Ola-Fadunsin (2020) identified helminth infections as a significant concern among cattle, noting substantial species diversity. In southern Ethiopia, Dembelo et al. (2023) documented cases of two or more gastrointestinal infections affecting cattle and concluded that deworming and effective management practices were necessary to eradicate gastrointestinal parasites. In eastern Uganda, parasitic worm burdens were reported as significantly high in cattle, with the situation worsening in small ruminants such as goats and sheep, where kid mortality rates have reached 40% (Namutosi et al., 2019). In central Uganda, approximately 56% of animals infected with helminths were found to be shedding *Haemonchus contortus* eggs (Nsereko et al., 2015). A study conducted by Kalule et al. (2023) reported a 63% prevalence of *Haemonchus contortus* adult worms in goats from selected districts in Uganda. This parasite, located in the gastrointestinal tracts of farm animals, causes anorexia, diarrhea, emaciation, and anemia, which can ultimately result in the death of the animal (Wright et al., 2018; Tiele et al., 2023).

The government of Uganda, through the Ministry of Agriculture, Animal Industry, and Fisheries (MAAIF), has recommended several interventions such as the use of chemotherapeutic drugs and grazing management practices to reduce helminth burdens in livestock, thereby improving productivity. The most recommended intervention is the use of

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imported wide-spectrum veterinary drugs such as Albendazole, Mebendazole, and Ivermectin. However, these veterinary drugs are costly for most farmers and are frequently unavailable in remote areas. Moreover, Aremu et al. (2012) noted that helminthiasis remains a persistent issue, particularly for poor livestock farmers.

As an alternative, some farmers rely on medical plants such as *Azadirachta indica*, *Tithonia diversifolia*, *Phytolacca dodecandra*, and *Vernonia amygdalina*, using indigenous knowledge to control helminths. These plants have been reported as effective (Bizimenyera, 2007; Nalule et al., 2011; Matovu et al., 2020). Therefore, in this study, the potency of two plant extracts of *Azadirachta indica* (*Azaradichta indica*; Neem) and *Tithonia diversifolia* (*T. diversifolia*; Mexican sunflower) on *Haemonchus contortus* were investigated. The main objective was to determine the phytochemical composition and *in-vitro* anthelminthic activity of the ethanolic extracts of *Azadirachta indica* and *Tithonia diversifolia* on *Haemonchus contortus.*

MATERIALS AND METHODS

Ethical approval

The study was approved by the Student Research Review Committee of the College of Veterinary Medicine, Animal Resources, and Biosecurity at Makerere University, Uganda. The *Haemonchus contortus* worms used in this experiment were collected from the abomasa of slaughtered goats. Permission to collect the samples was obtained from the management of Kalerwe abattoir through the Makerere University College of Veterinary and Biosafety Sciences Laboratory.

Plant sample collection

Leaves of *Tithonia diversifolia* and *Azadirachta indica* were collected in April 2022 from Kamu sub-county, Bulambuli district in Eastern Uganda. The area lies along 1° 17' 49" N and 34° 19' 5" E, approximately 1300 meters above sea level, and receives a bi-modal rainfall pattern, with wet months from April to October and dry months from July to August and December to February. The plant parts were identified by the Department of Botany Herbarium at Makerere University as S01-*Azadirachta indica* and S02-*Tithonia diversifolia*.

Extract preparation

Leaf samples of *Tithonia diversifolia* and *Azadirachta indica* (2.5 kg each) were air-dried for three weeks at an average room temperature of 22 °C. The air-dried plant samples were pulverized using a laboratory Brook Crompton electric grinder (UK) to pass through a 1mm screen. A total of 200 g of each ground sample was then added to 1000 ml of 70 % ethanol at room temperature. The mixtures were left to stand for three days with periodic shaking, following the study of Olukotun et al. (2018). At the end of the 3-day extraction period, the mixtures were filtered twice, first through cotton wool in a Burkard funnel and then using Whatman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator (CH-9230 Flawl / Schweiz, Germany) at 55°C until a constant volume was achieved. The plant extracts were dried in an oven set at a temperature of 55°C to a constant weight and then refrigerated at 4°C for the subsequent phytochemical analyses and anti-helminthic profiling.

Phytochemical screening tests

The ethanolic extracts of *Tithonia diversifolia* and *Azadirachta indica* were screened for various phytochemicals, including saponins, tannins, reducing sugars, starch, alkaloid salts, anthracenosides, anthocyanosides, coumarins, flavonosides, and steroid glycosides, as described by Das et al. (2018). Tests for the presence of saponins, tannins, reducing sugars, alkaloid salts, and anthracenocides were conducted using Frothing, Ferric chloride, Fehling's, Mayer's reagent, and Ammonia solution tests, respectively. The presence of starch was tested using an iodine solution while coumarin derivatives were detected using an ammonia solution and ultraviolet (UV) light test. The presence of flavonoids and steroid glycosides was confirmed utilizing Shibata's reaction and Liebermann-Burchard's test, respectively. The level of presence of the phytochemicals was indicated by the intensity of color change: (++) for high abundance, (+) for low abundance, and (-) for absence.

Collection of *Haemonchus contortus*

Adult *Haemonchus contortus* worms were harvested by washing the abomasa of goats slaughtered in Kalwere abattoir using phosphate buffer solution (PBS) following a standard procedure described by Hade et al. (2022). The washed worms were immediately put in a flask containing PBS, maintained at a temperature of 37°C, and delivered to the parasitology laboratory at the College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University, Uganda, for further management and *in-vitro* evaluation.

In-vitro anthelminthic activity assessment

The mortality of adult *Haemonchus contortus* was assessed using plant extracts at various concentrations. A stock solution of 20 mg/ml was prepared for daily treatment by dissolving 2 g or 2000 mg (dry matter) of each extract in 100 ml of PBS. Four treatments (concentrations) of 2.5 mg/ml, 5 mg/ml, 10 mg/ml, and 20 mg/ml of extracts from *Tithonia diversifolia* and *Azadirachta indica* were prepared for each plant sample using serial dilution with PBS as a solvent following the procedures described by Pandey et al. (2018). Concentrations of 2.5 mg/ml, 5 mg/ml, 10 mg/ml, and 20 mg/ml for each of the extracts were prepared by dissolving 250 mg, 500 mg, 1000 mg, and 2 g of dried powder, respectively, in 100 ml of the PBS solvent. The negative and positive controls consisted of 10 ml of PBS and Albendazole 2.5 %, respectively. Albendazole 2.5% was used because it is the lowest commercially available concentration of the drug recommended by the National Drug Authority (NDA, 2023). Ten adult worms were put in Petri dishes containing each of the plant extract concentrations/treatments and control solutions, with three replicates for each treatment. The petri dishes were placed in an incubator set at a constant temperature of 37°C for 8 hours. Inhibition of movement of the helminths was a sign of worm death or paralysis. Movement of helminthic parasites was observed, and live and dead worms were tallied at 30-minute different time intervals. The time intervals were 30 minutes, 1 hour, 2 hours, 3 hours, and 8 hours. Worms that did not show any movement were picked from the plant extract concentrations and put in lukewarm PBS for 15 minutes. Thereafter, worms were observed and counted as live in case of restoration of any movement. Otherwise, they were considered dead when no motility was recorded (Coles et al., 1992; Aliyi Hassen et al., 2020).

Statistical analysis

Data were entered into the Microsoft Excel spreadsheet, transferred to SPSS software (version 21), and analyzed using descriptive statistics including means and standard deviations.

RESULTS

Phytochemical composition

Phytochemical screening tests showed that *Azadirachta indica* and *Tithonia diversifolia* contained several active compounds (Table 1). *Tithonia diversifolia* indicated higher levels of coumarins than *Azadirachta indica.* Starch, anthracenosides, and anthocyanosides were present in *Azadirachta indica* but absent in *Tithonia diversifolia.*

Effects of *Azadirachta indica* on *Haemonchus contortus*

The results indicated that the mean time to death of *Haemonchus contortus* decreased progressively as the concentration of *Azadirachta indica* increased (Table 2). Total mortality of *Haemonchus contortus* (100%) was achieved most rapidly at 2.1 hours with *Azadirachta indica* at a concentration of 20 mg/ml (Table 2).

Effect of *Tithonia diversifolia* on *Haemonchus contortus*

The mean time to death of *Haemonchus contortus* on exposure to different treatments /concentrations of *Tithonia diversifolia* also decreased progressively with increasing concentrations (Table 3). Total mortality of *Haemonchus contortus* (100%) was achieved after 2.55 hours of exposure to *Tithonia diversifolia* at a concentration of 20 mg/ml (Table 3). In comparison, the positive control, albendazole at a concentration of 2.5%, was highly effective and caused 100% mortality within 30 minutes, significantly outperforming all treatments /concentrations of the plant extracts.

Table 1. Phytochemical composition of ethanolic extract of *Azadirachta indica* and *Tithonia diversifolia*

Test	*Azadirachta indica*	*Tithonia diversifolia*
Saponins	+ (moderate)	+ (moderate)
Tannins	+++	+++
Reducing sugars	-	-
Starch	+	-
Alkaloid salts	+	+
Anthracenosides	++	-
Anthocyanosides	+	-
Coumarins	+	++
Flavonosides	++	++
Steroidglycosides	++	++

(+) presence; (-) absence

Table 2. Mean time to death of *Haemonchus contortus* exposed to *Azadirachta indica* extract

Items	Concentration				NC	PC
Treatment	**2.5 mg/ml**	**5.0 mg/ml**	**10.0 mg/ml**	**20.0 mg/ml**		
Mean time to death (hours)	3.55	2.85	2.20	2.10	0.0	0.5
Variance in time to death	8.358	3.447	2.344	2.266	0.0	0.0

NC: Negative control, PC: Positive control

Table 3. Mean time to death of *Haemonchus contortus* exposed to *Tithonia diversifolia* extract

Items	Concentration				NC	PC
Treatment	**2.5 mg/ml**	**5.0 mg/ml**	**10.0 mg/ml**	**20.0 mg/ml**		
Mean time to death (hours)	3.85	3.05	2.80	2.55	0.0	0.5
Variance in time to death	6.447	4.344	3.914	3.525	0.0	0.0

NC: Negative control, PC: Positive control

DISCUSSION

The phytochemical screening tests of the two plant extracts confirmed the presence of saponins, tannins, flavonoids, alkaloids, anthracenosides, anthocyanosides, coumarins, and steroid glycosides. Both *Azadirachta indica* and *Tithonia diversifolia* contained flavonoids and steroid glycosides, as well as equally high levels of tannins. These biochemical compounds exhibit pharmacological effects, including anti-helminthic (Aremu et al., 2012; Zenebe et al., 2022; Ukwa et al., 2023), anti-oxidant (Gama et al., 2014; Gulcin, 2020; Nyero et al., 2023) antimicrobial, and anti-inflammatory properties (Bizimenyera, 2007; Gonfa et al., 2023). Tannin extracts have been reported to possess anthelmintic properties (Kotze et al., 2009; Greiffer et al., 2022). Similarly, Ferreira et al. (2013) noted that the presence of phenolic compounds in *Annona muricata* contributed to its anthelmintic effects. Alkaloids are also known to act on the central nervous system (CNS) of parasitic worms, causing paralysis, and also function as antioxidants (Adak and Kumar, 2022).

The time required to kill *Haemonchus contortus* worms on exposure to different treatments of plant extracts decreased with increasing concentrations, ranging from 2.5 mg/ml to 20 mg/ml. The shortest helminth elimination time for *Azadirachta indica* was 5 hours at a concentration of 20 mg/ml, while ethanolic extracts of *Tithonia diversifolia* achieved total mortality of *Haemonchus contortus* in 6 hours. These findings align with those of Sakti et al. (2018), who reported that dry ground leaf powder of *Azadirachta indica* significantly reduced the egg count of helminths after 28 days of treatment, likely due to the death of the parasite. Similarly, Duarte et al. (2020) used *Tithonia diversifolia* leaf powder on sheep inoculated with *Haemonchus contortus* and reported that the plant extract caused a great reduction in the population of albendazole-tolerant infective larvae of *Haemonchus contortus.* The results of the study revealed that *Azadirachta indica* extract was more effective than *Tithonia diversifolia*, possibly due to high levels of tannins and alkaloids and the presence of anthracenosides and anthocyanosides, which were absent in *Tithonia diversifolia.*

CONCLUSION

The present study provided evidence that ethanolic extracts of *Azadirachta indica* and *Tithonia diversifolia* at a concentration of 20 mg/ml have anthelmintic effects on *Haemonchus contortus*. This finding offers an excellent opportunity for advances toward developing low-cost alternative natural anthelmintic compositions to fight worm parasitic infestations in livestock. Based on the results, more *in vivo* experiments are recommended to determine the effective dosage and toxicity levels of these plant extracts for helminth control.

DECLARATIONS

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Authors' contributions

Brian Britex Owoyesigire designed the experiment, supervised the collection of plant samples, and drafted the manuscript; Laban Buyi performed the experiment, and collected and analyzed data; Joachine Idibu and Terence Odoch supervised data analysis and experimental designs, and revised the draft manuscript; and Lawrence Owere edited the manuscript. All authors read and corrected the manuscript for final approval.

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Competing interests

The authors have no competing interests.

Ethical considerations

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Availability of data and materials

The data to support this study is available upon request from the corresponding author

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