Research Article:

ETHANOLIC EXTRACT OF THE LEAF OF *Moringa oleifera* Lam. AS A POTENTIAL ANTHELMINTIC AGENT AGAINST GASTROINTESTINAL NEMATODES IN GOATS

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ABSTRACT

Moringa oleifera Lam. has been used in different countries to treat parasitic infections in humans and livestock. This study was aimed to investigate the anthelminthic activity of ethanolic extract of Leaf of *Moringa oleifera* Lam. (EEMO) against gastrointestinal nematodes in goats. EEMO was prepared in 100% ethanol by cold percolation and qualitative tests were performed for phytochemical screening. Forty male goats (Egg Per Gram/EPG at least 100) were randomly selected and allocated equally into Negative control (N-Ctrl), *Moringa oleifera* ethanolic extract (MO-100), MO-200 and Positive control (P-Ctrl). The goats of MO-100, MO-200 and P-Ctrl groups received orally MO extract @ 100 mg/kg, 200 mg/kg and albendazole 10 mg/kg body weight, respectively whereas normal water was provided to the goats of N-Ctrl group. EPG count was assessed by Mc-Master technique followed by FECR% (Fecal egg count reduction percentage) on day 0, 3, 7, 14 and 28. Phytochemical screening revealed the presence of saponin and flavonoid. EPG decreased and FECR% increased in both extracts treated groups on the dose dependent manner on day 7-28. The highest FECR% were observed with MO-100 (77.03±7.54) and MO-200 (91.83±2.26) on day 28. Therefore, our study has shown that MO @ 200 mg/kg has sufficient anthelmintic activity against gastrointestinal nematodes in goat.

Keywords: Flavonoid, Mc-Master, percolation, phytochemical, saponin

INTRODUCTION

Gastrointestinal nematodes (GINs) in particular create the main hurdle to the successful production of small ruminants worldwide (Dey et al., 2020). Goats in Nepal had 69.14% prevalence of gastrointestinal nematodes (Ghimire & Bhattarai, 2019; Khanal et al., 2024) .GINs have been related to reduction in goat production due to their detrimental effects, which include inappetence, reduction in weight, hypoproteinemia, and other pathological issues that result in decreased productivity, retarded growth, and even death (Akhter et al., 2011). Furthermore, the lack of good veterinary facilities and the suitable environment of the tropics facilitate the invasion and propagation of nematodes. Thus, nematode infection is common in Nepal (Adhikari et al., 2017).

Strongyles (*Haemonchus, Oesophagostomum, Trichostrongylus, Bunostomum*), *Strongyloides*, and *Trichuris* species are the major gastrointestinal nematodes in goats (Khanal et al., 1990). Goats infect gastrointestinal (GI) nematodes mostly by ingesting infective larvae from contaminated pastures, water, or housing. Direct, involving the fecal-oral route, is the most common mode of transmission (Tariq et al., 2010). Most gastrointestinal nematodes (GINs) share a similar life cycle and are typically oviparous, producing characteristic eggs. While

nematode life cycles may be direct or indirect, economically important GINs parasites of small ruminants generally have a direct life cycle without intermediate hosts (Abebe et al., 2018). Adult worms reside in the host intestine, reproduce, and their eggs are excreted in feces. Under suitable temperature and humidity, eggs hatch into first-stage larvae (L1), which develop into second-stage larvae (L2) and feed on bacteria. L2 then moult into infective third-stage larvae (L3), which migrate onto vegetation. These L3 larvae are ingested during grazing by sheep or goats. Inside the host, L3 develop into L4 within 2–3 days and later mature into adults within 10–14 days (M. Ahmed, 2010).

Different anthelmintic drugs are currently used to control nematode infections; however, their improper and indiscriminate use not only resulted in the emergence of anthelmintic resistance (Fissiha & Kinde, 2021) but also cause anthelmintic residues to dairy products and goat meat (Jedziniak et al., 2015). Moreover, anthelminthic drugs are expensive and cause the several adverse effects (Bagheri et al., 2004) ranging from mild, transient side effects like gastrointestinal distress (nausea, vomiting, abdominal pain, diarrhea), dizziness, headache, and fatigue to severe reactions like allergies, hepatotoxicity and Nephrotoxicity (Grover et al., 2001). Hence, there is an urgent need to develop efficacious, non-toxic, and cost-effective plant-derived anthelmintic alternatives.

Plant based anthelmintic activity is primarily resulting from the presence of secondary metabolites, including alkaloids, flavonoids, terpenes, glycosides, saponins, and tannins (Manjusa & Pradeep, 2022). These bioactive components use a variety of mechanisms to produce their anthelmintic effects, including damage to the parasite's intestine, disruption of sodium and potassium ion transport, acetylcholinesterase inhibition, interference with phosphorylation and energy metabolism, and suppression of nutrient absorption in helminths (Manjusa & Pradeep, 2022). Even at low concentrations, plant extracts have shown larvicidal and ovicidal properties against nematodes (Rates, 2001; Váradyová et al., 2018). Furthermore, plant-based extracts are widely available, cost-effective, and generally associated with fewer side effects (Nasim et al., 2022), causing them viable substitute treatments for nematodiasis. Till date, numerous herbal formulations have been developed and utilized as anthelmintic drugs (French, 2018; Kamaraj & Rahuman, 2011; Spiegler et al., 2017). Recent information regarding an inventory of anthelmintic plants across worldwide was established (H. Ahmed et al., 2023).

Moringa oleifera Lam. is a perennial, Angiospermic plant that is commonly referred to as the "drumstick" or 'horseradish' tree. The most extensively grown species, *Moringa oleifera* Lam., is indigenous to subtropical and tropical regions worldwide (Olson, 2002). It is commonly named as 'Sitalchini', Munga, Sahijan or Saijan in Nepal (Thapa et al., 2019). The bark, roots, flowers, seeds, leaves, and pods of *Moringa oleifera* Lam. possess the medicinal value (Pareek et al., 2023) and the leaves has long been utilized as a deworming treatment (Mehta et al., 2011). *Moringa oleifera* Lam. possess anti-diabetic, anti-bacterial, anti-fungal, anti-allergic, anti-leishmanial, anti-inflammatory, anti-oxidant, anti-cancer and anthelminthic property (Amin et al., 2024). Specifically, (Nigusie et al., 2023) intervened that the in vitro egg hatchability inhibition effect of ethanolic extract of Moringa oleifera leaf (EEMO) against Strongyles type egg of ovine. The invivo anthelminthic action of EEMO against goat gastrointestinal nematodes has not yet been studied.

Thus, the goal of the present study was to assess the in-vivo anthelminthic activity of EEMO at different dosages and its effect on the lowering of parasitic egg per gram (EPG) in goat feces.

RESEARCH METHODS

Collection and identification of plants

Fresh *Moringa oleifera* Lam. Leaves along with tender stem was collected on 2 March, 2025 from the Rupandehi district and was identified by the National Herbarium, Godavari, Lalitpur, Nepal (232/081-082).

Preparation of *Moringa oleifera* Lam. leaf's ethanolic extract (EEMO)

The 5 kg *Moringa oleifera* Lam. fresh leaves were cleaned, shade-dried (1 kg dry matter), then ground into powder using a electric blender. After that, the powder was macerated in ethanol (100%) for 48 hours while being shaken as frequently. The Whatman No. 1 filter paper was then used to filter the mixture. A rotary vacuum evaporator operating at lower pressure (200-300 mbar) and in between 40°C to 45°C was then used to concentrate the filtrates. The extracts were dried in a low-temperature hot air oven to eliminate any remaining alcohol at the Natural Products Research Lab, Thapathali Kathmandu Nepal, then kept on refrigerator at temperature 4°C for further use.

Phytochemical screening

The obtained extract (ethanolic) was screened for several phytoconstituents by using a number of qualitative chemical test at the Natural Products Research Lab (NPRL), Thapathali Kathmandu Nepal.

Test for alkaloids

Mayer's reagent (K_2HgI_4) was used to screen for alkaloid (Mayer's test). One milliliter of the plant extract was mixed with Mayer's reagent (few drops). Alkaloid components were thought to be present when a white to pale yellow precipitate developed (Kancherla et al., 2019).

Test for flavonoids

The sodium hydroxide test was used to determine whether flavonoids were present. Two milliliters extract were mixed with two drops of sodium hydroxide solution. Flavonoid molecules were validated by the emergence of a strong yellow color (Kancherla et al., 2019).

Test for phytosterol

Utilizing the Liebermann-Burchard reaction, phytosterols were found. Chloroform and extract were combined in equal amounts (2 ml each), and then 2 ml of Conc.Sulphuric acid was added. Three milliliters of acetic anhydride and diluted acetic acid (few drops) were then added to the mixture. The presence of phytosterols was indicated by the development of a distinctive bluish-green color (Ahmed et al., 2020).

Test for phenols

The ferric chloride assay was used to detect phenols. Two milliliters of a 5% neutral $FeCl_3$ solution were added to one milliliter of extract. The presence of phenolic components was indicated by a green to bluish coloring (Kancherla et al., 2019).

Test for saponins

The foam test was use to confirm saponins. Five milliliters of distilled H_2O were added on around 0.5 milliliters of extract, and the mixture was rapidly agitated. The presence of saponins was confirmed by the stable froth's persistence (Dubale et al., 2023).

Test for proteins

The xanthoproteic reaction was used to assess the protein content. A few drops of conc. nitric acid (HNO_3) were added on one milliliter of extract. The appearance of a yellow colour indicated the presence of proteins (Ali et al., 2018).

Test for carbohydrates

Molisch's test was used to assess carbohydrates. Two milliliters extract were mixed with a few drops Molisch's reagent, which is α-naphthol diluted in ethanol. The test tube's side was then meticulously lined with layers of concentrated sulfuric acid. The presence of carbohydrates was confirmed by the development of a violet ring at the interface between these two layers (Kancherla et al., 2019).

Test for glycosides

To find glycosides, test Keller–Killiani was applied. Several drops of ferric chloride and 0.5 milliliter of acetic acid was added to 2 milliliters of extract. One milliliter of Conc.sulfuric acid was cautiously added along the inner wall of test tube. The appearance of strong blue coloration on the junction between these two liquid layers confirmed glycoside (Kancherla et al., 2019).

Test for reducing sugar

The Fehling test reaction was done. One milliliter of Fehling's A and B solutions were combined on the test-tube for this test. This mixture is then brought to a boil for one minute. Two milliliters of extract were then added. The presence of reducing sugar is indicated by the emergence of the red brick precipitate (Das et al., 2014).

Experimental animal

Goat flock raised on semi-intensive system (grazed 6-8 hours/day) that were infected with GINs were screened using the direct smear and the fecal floatation methods as mentioned by Soulsby (1968) . Mc-Masters technique(modified) was used to calculate EPG. For experimental trial, forty male Boer goats (Age: 0.5-1 year) with EPG= 100(at least) were chosen. Since, experiment might use preselected larvae, sampled animals for trial had stayed away from anthelmintic medication for the previous two months.

Experimental design

A completely randomized design (blinded/double-control) was used for the study. The 40 male goats(N= 40) utilized on the study were infected naturally with GINs. The goats were divided to four groups at random: the Positive Control (P-Ctrl), Moringa oleifera extract (MO-100), MO-200, and Negative Control (N-Ctrl). Ten goats were in each group. The investigation was conducted from December 2025 to January 2026 at the Chandragiri Goat Farm in Chitwan, Nepal.

The Treatment Regimen used on this research has been mentioned below.

- **Negative Control (N-Ctrl) Group**: Goats were provided with 100 ml normal drinking water
- ***Moringa oleifera* Ethanolic Extract (MO-100) Group**: Goats received EEMO@100 mg/kgbwt. orally once (drench by mixing in 100 ml normal drinking water)
- ***Moringa oleifera* Ethanolic Extract (MO-200) Group**: Goats received EEMO @200 mg/kgbwt. orally once (drench by mixing in 100 ml normal drinking water)
- **Positive control (P-Ctrl) Group:** Goats were treated with Albendazole tablet @10 mg/kgbwt. orally once

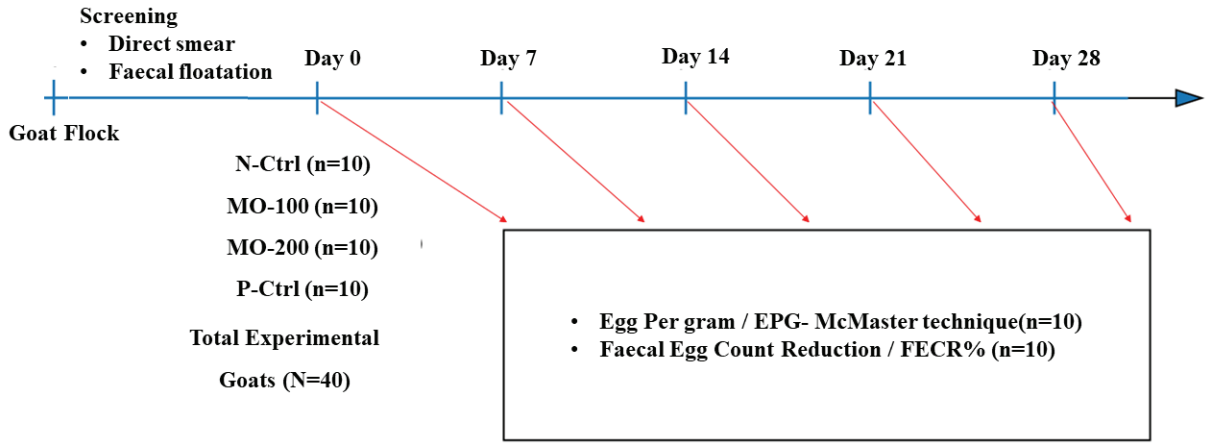

Fig. 1. Experimental Scheme of Research

Day0 was regarded as the experiment's beginning day. On Day0 (extract and anthelminthic medication pretreatment), 7, 14, 21, and 28, Three gram fecal samples were collected from each group of goats by introducing a finger inside the rectum.

EPG was assessed via Mc-Masters egg counting technique on Day0-28.

For this technique, using a mortar and pestle, three grams fecal sample were homogenized and 42 ml saturated NaCl (33%) solution (Specific gravity 1.20) was then added to grounded fecal samples. Three 15 ml centrifuge tubes were filled with the mixture. After that, a pipette was used to transfer about 0.15 ml of the liquid onto a McMaster slide @ALCON, after that wait for 5 mins. Then, microscope (10x) was used to count the eggs.

The EPG was estimated as:

EPG = 50× Eggs counted on both Two chambers

Then, Fecal Egg Count Reduction Percentage (FECR%) was calculated by following formulae on day7- 28.

FECR (%) = (Pre-treatment EPG – Post-treatment EPG) × 100 / Pre-treatment EPG

Using the standard WAAVP (World Association for the Advancement of Veterinary Parasitology) guidelines, anthelmintic activity of EEMO was screened on in-vivo against goat GINs (Coles et al., 1992), which was carried out at the Veterinary Pharmacology Lab under Department of Veterinary Surgery and Pharmacology at AFU, Rampur.

Statistical analysis

The Mean ± SD is used to present the data. The SPSS information system for Windows (SPSS V 27, SPSS Institute Inc. USA) @ Release on June 16, 2020 was used to conduct statistical analyses. Data of the EPG count and FECR% were analyzed through One-Way ANOVA followed by Fishers Least Significant Differences (LSD) for multiple mean comparisons. p-values less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Table 1. Phytochemical screening on *Moringa oleifera* Lam. Leaf's ethanolic extract

Active ingredients	Results
Alkaloids	-
Flavonoids	+
Phytosterol	-
Phenols	-
Saponins	+
Proteins	-
Carbohydrates	-
Glycosides	-
Reducing sugar	-

Phytochemical screening on EEMO detected the presence of flavonoids and saponins. These findings of the study are consistent with earlier studies (Abdulkadir et al., 2015; Khalid et al., 2023). Flavonoid and saponins are responsible for antihelminthic activity (N. Ali et al., 2011; Manjusa & Pradeep, 2022; Symeonidou et al., 2018). Saponins shows the anthelmintic action(at 30 mg/ml) through the acetylcholinesterase inhibition, resulting on worm paralysis and death (N. Ali et al., 2011). Flavonoid (at 4-12%) showing anthelminthic activity through phosphorylation reaction blockade resulting energy production inhibition which ultimately lead to the death of worms (Santos et al., 2018; Symeonidou et al., 2018).

Table 2. Egg Per Gram (EPG) in various treatment group from Day 0-28

Day	N-Ctrl	MO-100	MO-200	P-Ctrl	p-value
0	3530±512.72	3440±314.28	3450±206.82	3570±238.28	0.79
7	4320±596.84	2820±316.40**	2310±166.33**##	2980±228.76**++	0.00
14	4595±634.40	2195±321.84**	1305±140.33**##	2265±298.18**++	0.00
21	5000±645.49	1230±380.20**	725±113.65**##	1300±391.57**++	0.00
28	5490±613.64	800±300.92**	280±71.49**##	875±338.50**++	0.00

**p<0.01; *p<0.05 MO-100/MO-200/P-Ctrl versus N-Ctrl; ## p<0.01; #p<0.05 MO-200/P-Ctrl versus MO-100; ++ p<0.01 MO-200 versus P-Ctrl

On Day 0, EPG wasn't varied between treatment groups(P=0.79). EPG was varied in between treatment groups on Day7-28(P=0.00). EPG was lower(p<0.01) in MO-100 and MO-200 and P-Ctrl in comparison with N-Ctrl on Day 7-28. Additionally, EPG was lower(p<0.01) in MO-200 in compared with that of MO-100 and P-Ctrl group on these days.

Table 3. FECR% in various treatment group from Day7-28

Day	N-Ctrl	MO-100	MO-200	P-Ctrl	p-value
7	-22.57±5.66	18.06±4.70**	32.93±4.93**##	16.55±2.21**++	0.00
14	-30.39±6.86	36.43±4.25**	62.16±3.63**##	36.23±9.68**++	0.00
21	-42.11±8.80	64.69±8.72**	79±2.97**##	63.18±12.05**++	0.00
28	-56.30±8.61	77.03±7.54**	91.83±2.26**##	75.18±10.19**++	0.00

**p<0.01; *p<0.05 MO-100/MO-200/P-Ctrl versus N-Ctrl; ## p<0.01; #p<0.05 MO-200/P-Ctrl versus MO-100; ++ p<0.01 MO-200 versus P-Ctrl

The FECR% varied (P= 0.00) in between the N-Ctrl, MO-100, MO-200 and P-Ctrl on Day7-28. FECR% was markedly higher ($p<0.01$) in MO-100, MO-200 and P-Ctrl group in compared with the N-Ctrl group on Day 7- 28. FECR% was remarkably higher($p<0.01$) in MO-200 than that of MO-100 and P-Ctrl on these days.

EPG was reduced and FECR% was elevated on both extracts treated group in dose dependent manner/concentration dependent manner on day 7-28. That might be because of the extract's saponins have concentration- and dose-dependent inhibitory effects on nematodes eggs (Maestrini et al., 2020). Additionally, this is due to the dose-dependent nematocidal activity of flavonoids (Karakoti et al., 2025).

The highest FECR% were observed with MO-100 (77.03±7.54) and MO-200 (91.83±2.26) on day 28. So, both extract dose was efficacious in reduction fecal egg count. The 100 mg/kg extract efficacy is similar to albendazole@10mg/kg wt. however, 200 mg/kg extract was more effective than albendazole at 10 mg/kg weight.

This study illustrates the EEMO's dose-dependent antiparasitic effect. According to WAAVP guidelines, a reduction in fecal egg counts of 90% or more is considered highly efficient, while a reduction of 80% is considered sufficient (Githiori et al., 2006). EEMO decreased fecal egg counts by 91.83% at dose rate 200 mg/kg bwt. Given these recommendations, it is possible to hypothesize that EEMO@200 mg/kg bwt has sufficient anthelmintic activity and could be a good alternative to conventional anthelmintic in goats.

CONCLUSION

Phytochemical screening of EEMO showed the flavonoids and saponins. EPG was decreased and FECR% was increased in both extracts treated groups on the dose-dependent manner/concentration-dependent manner on day 7-28. The highest FECR% were observed with MO-100 (77.03±7.54) and MO-200 (91.83±2.26) on day 28. Therefore, MO @ 200 mg/kg bwt. has sufficient amount of anthelmintic action against GINs in goat.

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AUTHOR CONTRIBUTIONS

AP: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing; **SN:** Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing; **UP:** Investigation; **ST:** Resources, Funding acquisition, Writing – review & editing, Supervision; **MKS:** Resources, Funding acquisition, Writing – review & editing, Supervision.

CONFLICT OF INTEREST

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 paper.

ETHICAL APPROVAL AND PERMITS

This herbal extract dose is safe to the goat. Fecal samples collected non-invasively and authors declare that there is no any violation of animal welfare issue.

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