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Ethanolic Extract of the Root of *Asparagus Racemosus* as a Potential Anthelmintic Agent against Gastrointestinal Nematodes in Goats

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Abstract

Background: *Asparagus racemosus* (AR) plant was traditionally used as deworming remedy. The present study was aimed to investigate the anthelminthic activity of ethanolic extract of AR root (EEAR) against gastrointestinal nematodes in goats.

Methods: The EEAR were prepared in 100% ethanol by cold extraction process. Then, phytochemical screening of the extract was done by several qualitative tests. A total 40 male goats (EPG at least 100) were allocated equally randomly in AR-100, AR-200 and Positive control (P-Ctrl) and Negative control (N-Ctrl) groups. The goats of AR-100, AR-200 and P-Ctrl groups received EEAR at the rate of 100 mg/kg, 200 mg/kg and albendazole 10 mg/kg, respectively. However, normal water was provided to N-Ctrl group. EPG count was assessed by modified Mc-Master technique on day 0, 3, 7, 14 and 28 and FECR% were calculated on these days. Data of EPG count and FECR% were analyzed using One-Way-ANOVA followed by LSD for multiple mean comparisons by using SPSS.

Results: Phytochemical screening of EEAR revealed the presence of phytosterol, terpenoids, saponin, glycosides, carbohydrate and reducing sugar. The EPG count was decreased in both extract treated group on the dose dependent manner on day 7, 14, 21 and 28. The FECR% on the extract treated group was increased on dose dependent manner on day 7, 14, 21 and 28. The

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100 mg/kg bwt and 200 mg/kg bwt dose of extract showed the maximum FECR% 73.05±2.90 and 87.18±5.41 respectively on day 28.

Conclusion: The 200 mg/kg bwt dose of EEAR has sufficient anthelmintic activity against nematodes in goat.

Novelty: There was no research on anthelminthic efficacy of EEAR against gastrointestinal nematodes in goat. This research is novel approach for controlling the gastrointestinal nematodes in goat by using the phytoconstituent of AR root. This study scientifically contributes to the animal health and sustainable goat farming.

Keywords: *Asparagus racemosus,* anthelminthic activity, ethanolic extract, goat, nematodes

Introduction

Parasitic diseases, especially gastrointestinal nematodes (GINs) are a major constraint on profitable small ruminants' production worldwide (Dey et al., 2020). In Nepal, prevalence of gastrointestinal nematodes in goats was 69.14% (Khanal et al., 2024). Gastrointestinal nematodiasis is associated with high morbidity, adversely affecting growth/production and under the severe condition can lead into death in goat (Belga et al., 2024).

Different antihelmintic drugs are often used presently for control of nematodes, but their inappropriate use has resulted the antihelminthic drug resistance (Rojas-Morales et al., 2021) and causing the presence of drug residues in meat for human consumption (Moreno Torrejon & Lanusse, 2017). Additionally, these therapies are expensive and exhibit the numerous undesirable side effects (Bagheri et al., 2004). Hence, there is a great need for the development of nontoxic, cost effective and natural herbal antihelminthic alternatives.

Notably, antihelmintic activity of plants is due to its secondary metabolites like terpenes, glycosides, saponins, flavonoids, tannins and alkaloids (Manjusa & Pradeep, 2022). These active compounds show the antihelminthic activity through various mechanisms such as damaging of intestine, disturbance of sodium and potassium ions transportation, inhibition of acetylcholinesterase, blocking the phosphorylation reaction/ energy production, inhibiting nutrient absorption of helminth (Manjusa & Pradeep, 2022). Low concentration of plant extract can cause the ovicidal and larvicidal activity against nematodes (Rates, 2001; Váradyová et al., 2018). These extracts are abundantly available, inexpensive and cause minimal side effects (Nasim et al., 2022), making them potential alternative therapeutics for the nematodiasis. So far, several herbal preparations have been devised for their uses as antihelminthic agents (French, 2018; Kamaraj & Rahuman, 2011; Spiegler et al., 2017). The details about an Inventory of Anthelmintic Plants across the Globe have been elucidated recently (H. Ahmed et al., 2023). The *Asparagus racemosus* is widely distributed in Nepal and other geographical domains of world (Hasan et al., 2016); (Alok et al., 2013). The flowers, fruits and roots of *A. racemosus* possess the medicinal value and its roots have been traditionally used as deworming remedy (Soren & Yadav, 2021). *Asparagus racemosus* poses the antibacterial, galactagogue, neuroprotective, antiulcer, anti-inflammatory, immunomodulatory, antioxidant (Javaid et al., 2022) and thereby antihelminthic property (Soren & Yadav, 2021; Vishwakarma & Kumar,

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2021). Specifically, Soren & Yadav (2021) intervened that the antihelminthic activity of Methanolic extract of *Asparagus racemosus* root against *Hymenolepis diminuta* (cestode) and *Syphacia obvelata* (nematode) in invitro and invivo study on rats. So far, no research has investigated the antihelminthic activity of ethanolic extract of *Asparagus racemosus* root against gastrointestinal nematodes in goats. Therefore, the present study was designed to evaluate the in-vivo antihelminthic activity of ethanolic extract of *Asparagus racemosus* root at various doses and its effect on reduction of parasite's egg per gram gram (EPG) faeces of goat.

Materials and Methods

Collection and identification of plants

Asparagus racemosus was collected between June to July, 2023 in its whole (adult stage) from the Kafle Community Forest of Lalitpur district and was identified by National Herbarium in Godavari, Lalitpur, Nepal (505/079-080).

Preparation ethanolic extract of tuber root of *Asparagus racemosus*

The fresh clean roots were washed, shade dried and crushed using electric blender to make it fine powder. Then the powder was macerated in 100% ethanol for 48 hr with timely shaking. After that, the solution was filtered through the whatman No.1 filter paper. Then, the filtrates were concentrated using a rotary vacuum evaporator under reduced pressure at a temperature of 40–45°C. Then the extracts were dried in low temperature hot air oven to remove excess alcohol and stored in the refrigerator at 4 ºC until further use.

Phytochemical screening

At the Natural Products Research Laboratory (Department of plant resources), the produced ethanolic extract was tested using several qualitative chemical tests to determine whether it contained different phytoconstituents or not.

Test for Alkaloids

Mayer test (Potassium mercuric iodide) was performed. In this test, Mayer's reagent(few drops) were mixed with 1ml of extract, then observed for white yellowish precipitation which indicates the alkaloid's presence (Kancherla et al., 2019).

Test for Flavonoids

NaOH test was performed. In this test, two drops of NaOH were added to extract (2 ml). Formation of yellow color shows the flavonoid's presence (Kancherla et al., 2019).

Test for phytosterol

Liebermann Burchard test was performed. In this test, 2ml chloroform is added to extract(2 ml) and then conc. H2SO4(2 ml) is also added. On this solution, few drops dil.acetic acid and acetic anhydride(3 ml) were added. The emergence of bluish green color indicates the phytosterol's presence (Z. Ahmed et al., 2020).

Test for Terpenoids

Terpenoid test was performed. In this test, extract (5 ml) was mixed with 2 ml chloroform (CHCl3) and Conc. Sulfuric acid (3 ml). The appearance of the reddish-brown color indicates the terpenoid's presence (Z. Ahmed et al., 2020).

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Test for phenols

Ferric chloride (FeCl3) test was performed. In this test, 1ml of Extract is combined with and 2ml of 5% neutral FeCl3 solution. The presence of greenish bluish forms denotes the phenol's presence (Kancherla et al., 2019).

Test for saponins

Foam test was performed. In this test, extract(0.5ml) and distilled water (5 ml) were mixed and shaked. Then, the foam formation indicates the saponin's presence (Dubale et al., 2023).

Test for proteins

Xanthoproteic test was performed. In this test, extract(1 ml) was combined with conc. HNO3(few drops) and the appearance of yellow color confirmed the protein's presence (S. Ali et al., 2018).

Test for carbohydrates

Molisch's test (alpha-Naphthol in ethanol) was performed. In this test, few drops of Molisch's reagents were mixed to Extract(2ml). Later, Conc. H2SO4(few drops) were incorporated. The formation of violet ring in between the two liquid indicates carbohydrate's the presence (Kancherla et al., 2019).

Test for glycosides

Keller killiani test was performed. In this test, acetic acid (0.5 ml) and ferric chloride (2-3 drops) were mixed to extract (2 ml). Later, Conc. H2SO4(1ml) was added on the wall of test tube. The formation of deep blue coloration in between the two liquid indicated the glycoside's presence (Kancherla et al., 2019).

Test for reducing sugar

Fehling's test was performed. In this test, 1 ml of Fehling's A and Fehling's B solution were mixed in the test tube. Then, this solution is boiled for a minute. Then extract (2 ml) was added. The appearance of the red brick precipitation shows the reducing sugar's presence (Das et al., 2014).

Test for tannin

In this test, 10% FeCl3(3-4 drops) were mixed to 2ml extract, blue color was seen for gallic tannins and the green color was seen for the presence of catechol tannin (Sharma et al., 2020).

Experimental animal

Goat flock reared in semi-intensive management system (grazed for 6-8 hours/day) infected naturally with gastrointestinal nematodes were screened by using direct smear method and fecal floatation method as described by Soulsby (1968), additionally Eggs per Gram (EPG) was calculated using modified Mc Masters technique. Altogether 40 male goat (age :6month to 1years) having EPG at least 100, were chosen for experiment. The animals sampled for the study had not received any anthelmintic medications in the 2 months before because the experiment could involve pre-selected larvae.

Experimental design

The research was performed as completely randomized blinded double control design. The study used male goat (N=40) infected naturally with gastrointestinal nematodes. The goats

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were allocated randomly in Negative control (N-Ctrl), *Asparagus racemosus extract* (AR-100), AR-200 and Positive control (P-Ctrl) groups. In each group contained ten goats. The study was carried out at Chandragiri Goat farm, Chitwan, Nepal from January 2025 to February 2025. The study followed the small animal welfare guidelines and experiment was approved by ethical committee of Nepal Veterinary Council (Permission No. 213/2081-82).

The treatment protocol used in this study has been outlined below.

- **Negative control (N-Ctrl)** group: Goats were non-medicated but availed distilled water
- ***Asparagus racemosus* ethanolic extract (AR-100)** group: Goats received ethanolic extract of tuber root of *Asparagus racemosus* @100 mg/kg b.wt. orally once
- ***Asparagus racemosus* ethanolic extract (AR-200):** Goats were was given ethanolic extract of tuber root of *Asparagus racemosus* @200 mg/kg b.wt. orally once
- **Positive control (P-Ctrl) group:** Goats were medicated with Albendazole tab @ 10 mg/kg b.wt. orally once

The scheme of the experiment is represented in Figure.

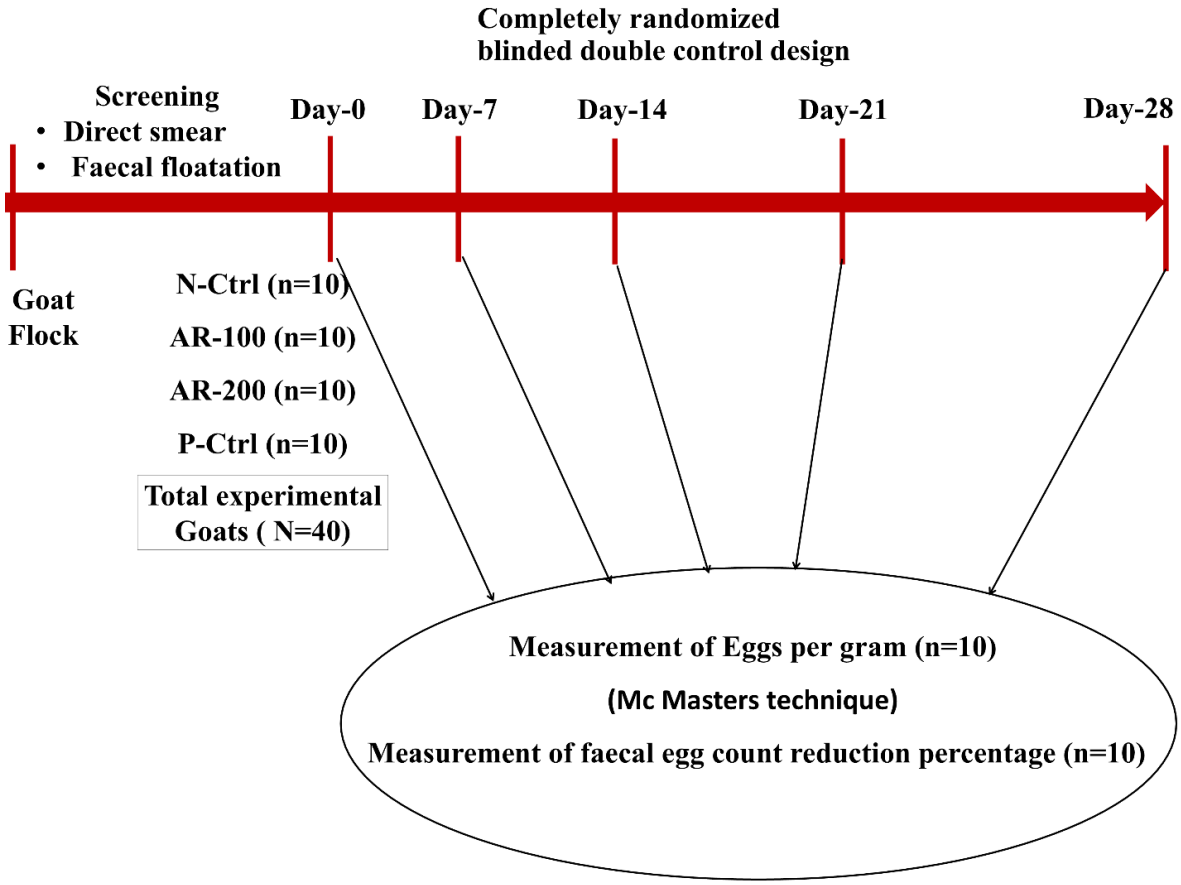

Figure 1: Scheme of experiment

Starting day of experiment was considered as day 0. Faecal samples were collected from all goat of all group on day 0 (pretreatment of extract and antihelminthic drug), day 7, 14, 21 28 by inserting the finger into rectum.

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Eggs per gram (EPG) was calculated by using the MacMasters egg counting technique on day 0, 7, 14, 21 and 28.

In this method, about 3 gm of sample were ground using mortar and pestle then the grounded samples were poured with 42 ml clean and fresh water. The mixture was placed in 3 centrifuge tubes (14 ml in each tube) and centrifuged for 2 minutes at 2000 rpm. The supernatant was removed and NaCl solution was added before straining. About 0.15 ml of the mixture was then placed on a Macmaster slide using a pipette and covered with a clean cover slip. Then the counting of eggs was done using a microscope. The EPG was calculated as:

EPG: Number of eggs counted in 2 chambers multiplied by 50

Then, Faecal egg count reduction percentage (FECR%) was determined using the following formula on day 7, 14, 21 and 28.

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

Screening of anthelmintic activity of the ethanolic extract of *Asparagus racemosus* root under invivo conditions against gastrointestinal nematodes of goat was done by using standardized protocol of WAAVP (World Association for the Advancement of Veterinary Parasitology) (Coles et al., 1992) ,which was conducted in laboratory of department of Parasitology, AFU, Rampur.

Statistical analysis

The data are presented as mean ± SD. Statistical analyses were performed using the SPSS information system for windows (SPSS V 27, SPSS Institute Inc. USA). Data of the EPG and FECR% were analyzed using One-Way ANOVA followed by LSD for multiple mean comparisons. The P-values <0.05 were considered significant.

Results

Table 1: Phytochemical screening of ethanolic extract of *Asparagus racemosus* tuber root

S.N.	Active ingredients	Results
1	Alkaloids	- ve
2	Flavonoids	- ve
3	Phytosterol	+ ve
4	Terpenoids	+ve
5	Phenols	- ve
6	Saponins	+ ve
7	Proteins	- ve
8	carbohydrates	+ ve
9	Glycosides	+ ve
10	Reducing sugar	+ ve
11	Tannin	-ve

Phytochemical screening of ethanolic extract of *Asparagus racemosus* (AR) root showed the presence of phytosterol, terpenoids, saponin, carbohydrate, glycosides and reducing sugar.

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Table 2: Egg per gram (EPG) in different treatment group from Day 0 to Day 28

	N-Ctrl	AR-100	AR-200	P-Ctrl	P-vale
Day 0	3105±495.22	3560±368.78*	3315±459.49	4510±298.88**##++	0.00
Day 7	3690±540.47	2745±337.02**	2300±461.88**#	3935±543.67##++	0.00
Day 14	3905±529.91	2080±348.16**	1110±282.64**##	2410±501.55**++	0.00
Day 21	4305±526.22	1070±184.39**	520±267.91**##	990±254.73**++	0.00
Day 28	4760±472.46	965±181.12**	445±246.58**##	565±261.45**##	0.00

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

The EPG count was significantly differed in between N-Ctrl, AR-100, AR-200 and P-Ctrl on day 0,7,14,21 and 28. On Day 0, EPG count was higher remarkably (P <0.01; 0.05) in AR-100 and P-Ctrl than that of N-Ctrl. Additionally, EPG count in P-Ctrl was higher(P<0.01) than AR-100 and AR-200 on Day 0. On Day 7, EPG count was lower(P<0.01) in AR-100 and AR-200 as compared to N-Ctrl. In addition to that the EPG count was lower(P<0.05) in AR-200 and higher(P<0.01) in P-Ctrl than that of AR-100 on this day. Similarly, the EPG count was higher(P<0.01) in P-Ctrl than that of AR-200 on Day 7. On day14, the EPG count in AR-100, AR-200 and P-Ctrl was remarkably (P<0.01) lower than that of N-Ctrl group. The EPG count on AR-200 was lower(P<0.01) as compared to AR-100 and P-Ctrl on day 14. On day 28, EPG was lower(P<0.01) in AR-100, AR-200 and P-Ctrl as compared to N-Ctrl group. Similarly, EPG Count in AR-200 and P-Ctrl was lower(P<0.01) in comparison with that of AR-100 on day 28.

Table 3: Faecal egg count reduction percentage (FECR%) in different treatment group from Day 7 to Day 28

	Day 7	**Day 14**	**Day 21**	**Day 28**
N-Ctrl	-19.18±6.78	-26.38±8.61	-39.70±11.44	-54.98±14.38
AR-100	22.99±2.65**	41.89±4.52**	70.10±2.71**	73.05±2.90**
AR-200	31.11±4.20**#	66.94±4.23**##	84.95±5.80**##	87.18±5.41**##
P-Ctrl	12.28±15.47**##++	46.30±13.00**#++	77.88±6.05**#+	87.43±5.87**##
P-Value	0.00	0.00	0.00	0.00

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

The FECR% was significantly differed between the N-Ctrl, AR-100, AR-200 and P-Ctrl on Day 7,14,21 and 28. On Day 7, FECR% was remarkably higher(P<0.01) in AR-100, AR-200

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and P-Ctrl group as compared with that of N-Ctrl group. FECR% was higher(P<0.05) in AR-200 and lower(P<0.01) in P-Ctrl as compared to AR-100 on day 7. The FECR% was remarkably lower(P<0.01) in P-Ctrl in comparison with that of AR-200 on day 7. On Day 14, FECR% was remarkably higher(P<0.01) in AR-100, AR-200 and P-Ctrl group as compared with that of N-Ctrl group. FECR% in AR-200 group was higher(P<0.01) than that of AR-100 and lower(P<0.01) than that of P-Ctrl group on Day 14. On Day 21, FECR% was remarkably higher(P<0.01) in AR-100, AR-200 and P-Ctrl group as compared with that of N-Ctrl group. The FECR% was higher (P <0.01; 0.05) in AR-200 and P-Ctrl as compared to AR-100 on this day. However, the FECR% in AR-200 is remarkably higher (P <0.05) than that of P-Ctrl group on Day 21. On Day 28, FECR% was remarkably higher(P<0.01) in AR-100, AR-200 and P-Ctrl group as compared with that of N-Ctrl group. The FECR% was higher (P <0.01) in AR-200 and P-Ctrl as compared to AR-100 on this day. However, there were no significant difference in FECR% of AR-200 and P-Ctrl group on day 28.

Discussion

Phytochemical screening of ethanolic extract of *Asparagus racemosus* (AR) root showed the presence of phytosterol, terpenoids, saponin, glycosides, carbohydrate and reducing sugar. These findings of this research are similar with previous study (Shevale et al., 2015). Phytosterol, terpenoids, saponin and glycosides are mainly responsible for anthelminthic activity (Manjusa & Pradeep, 2022). Phytosterols shows the anthelminthic activity by damaging the parasite's outer structure, leading to death (Lalthanpuii & Lalchhandama, 2020). Terpenoids produces the anthelmintic activities by the intestinal damage of parasite (Mukherjee et al., 2016). Saponins causes the anthelmintic activity through the inhibition of acetylcholinesterase and resulting the worm paralysis that leads to death (N. Ali et al., 2011). Glycosides have potent activity against different helminths (Manjusa & Pradeep, 2022). It causes potassium and sodium ions transportation disturbance of helminths and causing death of helminths (Hussein & El-Anssary, 2019).

The EPG count was decreased in both extract treated group on the dose-dependent manner in day 7, 14, 21 and 28. The FECR% on the extract treated group was increased on dose-dependent manner in day 7, 14, 21 and 28. This is due to terpenoids present in the extract causes the nematocidal activity against dose-dependent manner (Abdel-Rahman et al., 2013). Additionally, Saponins in present in this extract causes the inhibiting effects on nematodes eggs in a concentration/ dose-dependent manner Maestrini et al. (2020). Also, Phytosterol present in the extract causes the antinematodal effect in a dose-dependent manner (Manjusa & Pradeep, 2022). In addition to that, glycoside present in the extract inhibit the sodium and potassium ion transportation of helminths in dose dependent manner (Hussein & El-Anssary, 2019).

The 100 mg/kg bwt and 200 mg/kg bwt doses of extract showed maximum FECR% 73.05±2.90 and 87.18±5.41 respectively on day 28. So, both dose of extract was effective on faecal egg count reduction however the effectiveness of 200 mg/kg extract was higher which was equivalent to albendazole 10mg/kg wt. This study demonstrates the dose dependent

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antiparasitic activity of the extract. In accordance with the recommendations of WAAVP, a decrease in fecal egg counts of ninety-nine percent or more are regarded as highly efficient, while a decrease of eighty percent is regarded as sufficient (Githiori et al., 2006). Ethanolic extracts of *Asparagus racemosus* at dose 200mg/kgbwt reduced faecal egg counts by more than 87.18%. In light of these recommendations, it may be hypothesized that ethanolic extract of root of *Asparagus racemosus* 200mg/kg bwt has sufficient anthelmintic activity and might serve as a viable substitute for traditional anthelmintic in goats.

Conclusion and Recommendation

Phytochemical screening of ethanolic extract of *Asparagus racemosus* (AR) root showed the presence of phytosterol, terpenoids, saponin, glycosides, carbohydrate and reducing sugar. The EPG count was decreased in both extract treated group on the dose-dependent manner in day 7, 14, 21 and 28. The FECR% on the extract treated group was increased on dose dependent manner on day 7, 14, 21 and 28. The 100 mg/kg bwt and 200 mg/kg bwt dose of extract showed the maximum FECR% 73.05±2.90 and 87.18±5.41 respectively on day 28. So, it is concluded that only 200 mg/kg bwt dose of ethanolic extract of *Asparagus racemosus* root has sufficient anthelmintic activity against nematodes in goat. Further research is imperative to explore the pharmacological and toxicological evaluation of the extract.

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