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

Arjun Pandey
PhD Scholar
Department of Veterinary Surgery and Pharmacology, Faculty of Animal Science
Veterinary Science and Fisheries, Agriculture and Forestry University, Nepal
pandeyarjunvet@gmail.com

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Abstract

Asparagus racemosus (AR) is a medicinal plant of Liliaceae family, traditionally used as treatment for helminths. The present study was aimed to investigate the anthelmintic activity of aqueous extract of AR root against gastrointestinal nematodes in goats. The extracts of AR root were prepared by boiling in distill water followed by filtration in Whatman No.1 filter paper. Then, phytochemical screening of the extract was done by using several qualitative tests. Altogether 40 male goats having EPG at least 100 were chosen for experiment after the screening of naturally infected flock by direct smear and faecal flotation method. Then, total 40 male goats 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 oral administration of aqueous extract of AR root at a rate of 100 mg/kg, 200 mg/kg and albendazole 10 mg/kg, respectively. However, distilled water was provided to goats of N-Ctrl group. The EPG was assessed by modified Mc-Master technique on day 0, 3, 7, 14, and 28. Also, FECR% were assessed on day 7, 14, 21 and 28. Data of EPG and FECR% were analyzed using One-Way ANOVA followed by LSD for multiple mean comparisons by using SPSS. Phytochemical screening on this extract showed the presence of phytosterol, saponin, carbohydrate and reducing sugar. The EPG was decreased in extract treated group on the dose dependent manner on day 7, 14, 21 and 28. The FECR% is higher on both extract treated group on day 7. However, FECR% on the extract treated group was increased on dose dependent manner on day 14, 21 and 28. The extract at the dose rate 100 mg/kg bwt and 200 mg/kg bwt showed the maximum FECR% 59.05±0.73 and 88.82±4.94 respectively on day 28. So, it is

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concluded that aqueous extract of root of *Asparagus racemosus* at dose rate 200 mg/kg bwt has sufficient anthelmintic activity against gastrointestinal nematodes in goat. Further research is imperative to separate the possible anthelmintic component and understand its mechanism of action, bioassay-guided fractionation should be utilized for further evaluation of bioactive component.

Keywords: *Asparagus racemosus,* aqueous extract, root, anthelmintic activity, nematodes, goat

Introduction

Globally, gastrointestinal nematodes (GIN) are the main issue in goat production, particularly in tropical and subtropical nations (Santos et al., 2019). These parasites cause significant productivity losses, alter clinicopathology, and increase mortality (Cantacessi et al., 2012; Roeber et al., 2013). In Nepal, 69.14% goats were infected with gastrointestinal nematodes (Khanal et al., 2024).

Goats are highly vulnerable to GIN due to the weak immune system and faster capabilities of metabolizing the anthelmintic drug resulting reducing their efficacy (Hoste et al., 2010). Benzimidazoles, macrocyclic lactones and imidazothiazoles classes of anthelmintic drugs are often used presently for control of nematodes in goat (Alaro et al., 2023) but chronic use of the same anthelmintic class of medication, excessive treatment frequency, and insufficient anthelmintic dosage cause these parasites to develop anthelmintic resistance (Chandrawathani et al., 2004; Jabbar et al., 2006; Saddiqi et al., 2006). Additionally, these therapies are expensive and exhibit the numerous undesirable side effects (Bagheri et al., 2004). Also, these therapies increase the risks of residues in the meat, milk and the environment (Emery et al., 2016; Torres-Acosta et al., 2012). Hence, there is a great need for the development of nontoxic, cost effective and natural herbal anthelmintic alternatives.

Notably, several plants showed the anthelmintic activity of plants due to its secondary metabolites like terpenes, glycosides, saponins, flavonoids, tannins and alkaloids, through the multiple mechanisms (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, cause minimal side effects (Nasim et al., 2022) and eliminate the risks of drug residues in the meat, milk and the environment, making them potential alternative therapeutics for the nematodiasis. So far, several herbal preparations have been devised for their uses as anthelmintic 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

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anthelmintic property (Soren & Yadav, 2021; Vishwakarma & Kumar, 2021). Specifically, Soren & Yadav (2021) intervened that the anthelmintic 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 anthelmintic activity of aqueous extract of *Asparagus racemosus* root against gastrointestinal nematodes in goats. Therefore, the present study was designed to evaluate the in-vivo anthelmintic activity of aqueous extract of *Asparagus racemosus* root at various doses and its effect on reduction of parasite's egg per gram (EPG) faeces of goat.

Material 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 aqueous extract of tuber root of *Asparagus racemosus*

The fresh clean roots were crushed by using electric blender and boiled in 5-liter distilled water for 2 hrs. After that, the aqueous suspension was filtered into a different beaker using Whatman No.1 filter paper. Then, extract was dried in a water bath for 24 hrs. Then, aqueous extract was obtained. The obtained extract was stored at 4ºC for further use.

Phytochemical screening

The produced aqueous extract was tested using several qualitative chemical tests to determine whether it contained different phytoconstituents or not at the Natural Products Research Laboratory (Department of plant resources), Thapathali, Kathmandu, Nepal.

Test for Alkaloids

The Mayer test was done. In this test, 1 ml of extract was added with the two drops of potassium mercuric iodide (Mayer's reagent), and the presence of alkaloids was confirmed by observing for a white yellowish precipitate (Kancherla et al., 2019).

Test for Flavonoids

The NaOH test was carried out. In this test, two drops of sodium hydroxide were applied to extract (2 ml), flavonoid's presence is shown by an appearance of a yellow colour (Kancherla et al., 2019).

Test for phytosterol

Liebermann Burchard test was done. This test consists of adding 2 ml of chloroform to extract(2ml), followed by the addition of conc. H_2SO_4 (2 ml). After that, diluted acetic acid (few drops) and acetic anhydride (3 ml) were added to this mixture. The presence of phytosterols was denoted by the bluish green appearance (Z. Ahmed et al., 2020).

Test for Terpenoids

Terpenoid test was carried out. The 5 ml extract was added to conc. H_2SO_4(3 ml) and $CHCl_3$ (2 ml) for this test. The terpenoid's presence were indicated by formation of reddish-brown colour (Z. Ahmed et al., 2020).

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Test for phenols

For testing phenol, ferric chloride (FeCl3) test was carried out. In this test, 2 ml of a 5% neutral FeCl3 solution are mixed with extract (1 ml). The phenol's presence was proven by the development of greenish bluish forms (Kancherla et al., 2019).

Test for saponins

A foam test was conducted. The extract (0.5 ml) and distilled water (5 ml) were mixed together and shaked throughout this test. The presence of saponins was then verified by the foam that formed (Dubale et al., 2023).

Test for proteins

Xanthoproteic test was done. When the conc. HNO3(few drops) were added to extract(1 ml), a yellow color formed that suggesting the protein's presence (S. Ali et al., 2018).

Test for carbohydrates

The Molisch test was executed. The extract(2ml) was added with a few drops of Molisch's reagents (alpha-Naphthol in ethanol). A few drops of conc. H2SO4 were later added. Carbohydrates are present when the formation of violet ring in between two liquids (Kancherla et al., 2019).

Test for glycosides

The Keller-Killiani test was done. The extract (2 ml) was combined with acetic acid (0.5 ml) and ferric chloride (2-3 drops). Later, Conc. H2SO4(1ml) was applied along the test tube wall. Appearance of intense blue color in between two liquids, signifying the glycoside's presence (Kancherla et al., 2019).

Test for reducing sugar

The Fehling test was run. Fehling's A solution (1ml) and Fehling's B solution (1 ml) were mixed in a test tube. After that, mixture was boiled for one minute. Then, extract (2 ml) was then added. There is reducing sugar present when red brick precipitation forms (Das et al., 2014).

Experimental animal

Goat flocks raised in semi-intensive management systems (grazed 6–8 hours per day) and naturally infected with gastrointestinal nematodes were screened using direct smear and faecal flotation methods as described by Soulsby (1968). Additionally, the modified Mc Masters technique was used to estimate the eggs per gram (EPG). For the trial, 40 male goats (ages 6 months to 1 year) with an EPG at least 100 were selected. Because the experiment might involve pre-selected larvae, the animals sampled for the investigation had not taken any anthelmintic drugs in the two months prior.

Experimental design

The research was performed as completely randomized design. The study used male goat (N=40) infected naturally with gastrointestinal nematodes. The goats 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 November 2024 to December 2024. The treatment protocol used in this study has been outlined below.

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- **Negative control (N-Ctrl)** group: Goats were non-medicated but provided distilled water,
- ***Asparagus racemosus* aqueous extract (AR-100)** group: Goats received aqueous extract of tuber root of *Asparagus racemosus* @100 mg/kg b.wt. mixing with 100 ml distilled water orally (with the help of syringe) once
- ***Asparagus racemosus* aqueous extract (AR-200):** Goats were given aqueous extract of tuber root of *Asparagus racemosus* @200 mg/kg b.wt. mixing with 100 ml distilled water orally (with the help of syringe) once
- **Positive control (P-Ctrl) group:** Goats were medicated with Albendazole tab @ 10 mg/kg b.wt. orally once

Day 0 of the experiment was the day it began. A finger was inserted into the rectum, faecal samples were taken from every goat in every group on day 0 (pretreatment with extract and anthelmintic medication), day 7, day 14, day 21, and day 28.

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

In this technique, roughly 3 grams of the faecal sample were ground in mortar and pestle and 42 ml of fresh, clean water were then added to the ground samples. Three 14 ml centrifuge tubes were filled with this mixture, and it was centrifuged for 2 min. at 2000 rpm. Then, the supernatant was removed and a NaCl solution was added followed by straining. Then, using a pipette, 0.15 ml of the mixture was put on a Macmaster slide and covered with a clean cover slip. After that, a microscope was used to count the eggs (Panta et al., 2024). The EPG was calculated as:

EPG: Number of eggs counted in 2 chambers × 50

Then, **Faecal egg count reduction percentage (FECR%)** was calculated by using the formula mentioned below on day 7, day 14, day 21 and day 28.

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

Assessment of anthelmintic activity of the *Asparagus racemosus* root aqueous extract under in vivo 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.

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Results

Phytochemical screening

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

Table 1. Phytochemical screening of aqueous extract of *A. 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

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-value
Day 0	2920±374.31	3065±375.68	3015±439.72	3040±314.28	0.83
Day 7	3215±414.36	2615±319.76**	2315±338.33**#	2270±232.37**#	0.00
Day 14	3400±435.25	2315±286.79**	1505±189.22**##	1500±154.56**##	0.00
Day 21	3715±454.63	1840±219.59**	740±154.20**##	730±91.89**##	0.00
Day 28	4055±491.28	1255±157.14**	330±125.16**##	315±47.43**##	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*

The EPG wasn't varied between treatment groups on day 0 however, EPG was significantly differed between N-Ctrl, AR-100, AR-200 and P-Ctrl group on day 7, 14, 21 and 28. The EPG was lower (P<0.01) in AR-100, AR-200 and P-Ctrl in comparison to N-Ctrl group on day 7. Similarly, EPG is lower (P<0.05) in AR-200 and P-Ctrl as compared to AR-100 on this day. The EPG is lower (P<0.01) in AR-100, AR-200 and P-Ctrl in comparison to N-Ctrl group on day 14, day 21 and day 28. Also, EPG is lower (P<0.01) in AR-200 and P-Ctrl than that of AR-100 on these days. However, there was no significant difference of EPG between AR-200 and P-Ctrl groups.

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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	-10.09±0.65	-16.44±2.13	-27.32±3.21	-39.03±4.69
AR-100	14.67±0.45**	24.46±1.07**	39.93±0.93**	59.05±0.73**
AR-200	21.67±17.28**	49.71±5.47**##	75.31±4.90**##	88.82±4.94**##
P-Ctrl	25.31±0.72**##	50.63±1.44**##	75.95±2.16**##	89.65±0.95**##
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 AR-200/P-Ctrl versus AR-100*

The FECR% is significantly varied between treatment group on day 7,14, 21 and 28. The FECR% was higher (P <0.01) in AR-100, AR-200 and P-Ctrl in comparison to N-Ctrl group on day 7. The FECR% was high (P <0.01) in P-Ctrl than that of AR-100 on this day. Although, the FECR % is numerically higher on AR-200, there was no significant difference in FECR% of AR-100 and AR-200 on this day. Similarly, FECR% in AR-200 and P-Ctrl wasn't varied on this day. The FECR% was higher (P <0.01) in AR-100, AR-200 and P-Ctrl in comparison to N-Ctrl group on day 14, 21 and 28. The FECR% was high (P <0.01) in AR-200 and P-Ctrl than that of AR-100 on these days. However, there was no significant difference in FECR% between AR-200 and P-Ctrl groups.

Discussion

Phytochemical screening of aqueous extract of AR root revealed the presence of phytosterol, saponin, carbohydrate and reducing sugar which is consistent with the findings of previous study (Tinrat & Sila-asna, 2017). Tinrat & Sila-asna (2017) additionally found the flavonoid, triterpenoid, glycoside and alkaloid in the aqueous extract of asparagus racemosus root in distill water. This variation is attributed to the difference in plants part: solvent ratio used in the extraction process and plant sample obtained from different localities.

Saponin and Phytosterol are mainly responsible for antihelmintic activity (Cavalcante et al., 2016; Lalthanpuii & Lalchhandama, 2020). Saponins show their anthelmintic activity by inhibiting acetylcholinesterase and causing worm paralysis leading to death (N. Ali et al., 2011). Saponins are reported to have inhibitory activity on nematodes of animals (Cavalcante et al., 2016). Phytosterols shows the anthelmintic activity by damaging the parasite's outer structure, leading to death (Lalthanpuii & Lalchhandama, 2020).

The EPG was decreased in extract treated group on the dose dependent manner on day 7, 14, 21 and 28. The FECR% is higher on both extracts treated group on day 7. However, FECR% on the extract treated group was increased on dose dependent manner on day 14, 21 and 28. This is due to the saponin present in the extract destabilize nematode egg membranes making them more permeable followed by penetration into the eggs and damage their contents, preventing the nematode's larval development (Gomes et al., 2016). These findings are in consistent with the previous study (Maestrini et al., 2020) . Maestrini et al. (2020) reported that the saponins causes the inhibitory effects to the nematode's eggs on a concentration-dependent

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manner. Additionally, phytosterol present in the extract causes the antinematodal effect on a dose-dependent manner mechanisms (Manjusa & Pradeep, 2022).

The 100 mg/kg bwt extract dose showed maximum FECR% 59.05±0.73 and 200 mg/kg bwt extract dose showed maximum FECR% 88.82±4.94 on day 28, which is similar to albendazole group. 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 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). Aqueous extracts of *Asparagus racemosus* at dose 200mg/kgbwt reduced faecal egg counts by more than 88.82%. In light of these recommendations, it may be hypothesized that aqueous extract of root of *Asparagus racemosus* 200 mg/kg bwt has sufficient anthelmintic activity and might serve as a viable substitute for traditional anthelmintic in goats.

Conclusion

Phytochemical screening of aqueous extract of AR root revealed the presence of phytosterol, saponin, carbohydrate and reducing sugars. The EPG was decreased in extract treated group on the dose dependent manner on day 7, 14, 21 and 28. The FECR% is higher on both extract treated group on day 7. However, FECR% on the extract treated group was increased on dose dependent manner on day 14, 21 and 28. The extract at the dose rate 100 mg/kg bwt and 200 mg/kg bwt showed the maximum FECR% 59.05±0.73 and 88.82±4.94 respectively on day 28. So, it is concluded that aqueous extract of root of *Asparagus racemosus* at dose rate 200 mg/kg bwt has sufficient anthelmintic activity against gastrointestinal nematodes in goat.

Recommendations for further research

In-depth investigations into the mechanisms underlying the anthelmintic properties of aqueous extract of AR root are essential to elucidate how plant extract exerts its effects. Further research is imperative to separate the possible anthelmintic component and understand its mechanism of action, bioassay-guided fractionation should be utilized for further evaluation of bioactive component. This research shows that the both dose of plant extract exhibits the anthelmintic activity. Therefore, it is recommended to conduct further research using doses higher than 200 mg/kg bwt so that maximum doses of extract that show the maximum anthelmintic efficacy can be determined.

Transparency Statement: The author confirms that this study has been conducted with honesty and in full adherence to ethical guidelines.
Data Availability Statement: Author can provide data.
Conflict of Interest: The author declares there is no conflicts of interest.
Authors' Contributions: The author solely conducted all research activities i.e., concept, data collecting, drafting and final review of manuscript.

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