Original Research (2025) 2(2); 1 - 6
https://doi.org/10.22103/vcbr.2024.23657.1022

Shahid Bahonar University of Kerman

Veterinary and Comparative Biomedical Research

ORIGINAL ARTICLE

In Vitro Evaluation of the Anthelmintic Activity of *Rhus coriaria* (Sumac) Extract against *Marshallagia marshalli*

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Online ISSN: 3060-7663 **https://doi.org/10.22103/vcbr.2025.24623.1037**

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Article History

Received: *3 January 2025*
Revised: *18 March 2025*
Accepted: *12 May 2025*
Published: *7 July 2025*

Keywords

Marshallagia marshalli
Rhus coriaria
Anthelmintic Activity

Running Title

Anthelmintic Effect of Sumac on M. marshalli

Abstract

Marshallagia marshalli, a common parasite in domestic and wild ruminants in Iran, causes parasitic gastroenteritis. Chemical medicines like Levamisole are used to treat this infection, but drug-resistant infections have led to research on alternative anti-infective agents. This study investigated the anthelmintic properties of ethanol extract of sumac (*Rhus coriaria*) on *M. marshalli* in the laboratory. Three dilutions (25, 50, and 75 mg/ml) of the ethanolic extract of Sumac were prepared. Worms separated from the abomasum were exposed to the extracts at 37°C for up to 10 hours, and worm motility was evaluated hourly. Worms that did not move were considered dead, and the percentage of surviving worms was calculated. PBS buffer and levamisole (50 mg/ml) served as negative and positive controls, respectively. Results showed anthelmintic effects in all dilutions of the alcoholic extract compared to PBS buffer. However, there was no significant difference in worm survival compared to levamisole. The death rate of worms increased with higher concentrations of the extract and prolonged exposure time. In conclusion, sumac shows notable anthelmintic effects in laboratory conditions, providing a basis for further field research. Our study's main limitation is the inherent constraints of in vitro models, which may not fully replicate complex interactions within a living organism. Future researches should identify the active compounds in the extracts responsible for the scolicidal activities and conduct in vivo studies to assess their effectiveness and safety.

How to cite this article: *Soheila Fotoohi, Elham Mottaghi, Fatemeh Nouri, Saeid Reza Nourollahi Fard, Mehran Mesgari Abbasi. In Vitro Evaluation of the Anthelmintic Activity of Rhus coriaria (Sumac) Extract against Marshallagia marshalli. Veterinary and Comparative Biomedical Research, 2025, 2(2): 1 – 6. http://doi: 10.22103/vcbr.2025.24623.1037*

Introduction

Ruminants are a primary source of protein in human diets, and their parasitic infections are of significant concern. Among these, *Marshallagia marshalli* is the most common nematode in the digestive system of various ruminants (1). This parasite belongs to the Trichostrongylidae family, with adult worms residing in the abomasum and occasionally in the duodenum of sheep, goats, and camels (2). Parasite eggs are excreted with feces, and under suitable conditions, infectious larvae develop inside the eggs. With sufficient moisture, the larvae hatch and enter the ruminant's digestive system through grazing (3). The larvae molt in the rumen and then move to the abomasal gastric glands for further development (4). Animals previously infected show significant resistance to re-infection due to an activated immune system (5). The immune response in the digestive tract mucosa includes increased infiltration of lymphocytes, mast cells, eosinophils, and enhanced release of inflammatory mediators, often accompanied by mucosal edema and hyperemia (6). Diagnosis is based on clinical and laboratory findings, as well as the necropsy of suspected animals. Recent studies have shown that despite the effectiveness of anti-infective agents, the development of drug resistance is a serious concern. Previous studies have also reported resistance to anti-parasitic drugs, meaning that worms remain alive after treatment (6).

Sumac (*Rhus coriaria*) is one of the top 10 plant antioxidants, growing mainly in subtropical and temperate regions (7). Its properties include destroying cancer cells, relieving stomach pain, and reducing blood pressure. Sumac is beneficial for diabetes patients and reduces infertility in diabetic individuals (8). Additionally, it prevents gastric bleeding and acts as an astringent and stomach cleanser due to its high tannin content. Sumac prevents biliary issues, chronic diarrhea, and bloody diarrhea, and stops bleeding. It strengthens gums, aids in growth (9), helps reduce cholesterol and blood lipids (10), and treats hyperuricemia, gout (11), and rheumatism. Sumac also has antimicrobial properties effective against *Klebsiella* and *Staphylococcus* spp. (12). Moreover, it has an effect on hydatid cysts (13).

Considering the anti-infective properties of sumac reported in various studies, the current research aims to investigate its anthelmintic effect. Sumac can be used as an appropriate, less dangerous, and cheaper alternative treatment for Marshallagia infections as an anti-parasitic agent.

Materials and Methods

Preparation of Dried and Ground Material

To prevent the effects of molds, bacteria, enzymes, and chemical changes, sumac drying was done. Drying preserves the chemical compounds and makes it easier to grind and extract them. Sumac was placed in the shade at natural temperature for several days to dry completely. After drying, it was ground and then passed through a sieve.

Preparation of Ethanolic Extract

The ethanol soaking method was used to prepare the extract. For this purpose, 50 grams of sumac were soaked in 200 ml of ethanol as a solvent for 48 hours. Then, the soaked solution was filtered using a Buchner funnel connected to a vacuum. The filtered solution was concentrated by distillation for about 2 hours. The concentrated solution was placed in an oven at 40 to 50 °C to dry. The prepared extract was stored at 4 °C until use.

Preparation of Concentrations

To prepare three concentrations, 0.5, 1, and 1.5 grams of ethanolic extract were dissolved in 20 ml of PBS buffer to obtain concentrations of 25, 50, and 75 mg/ml, respectively.

Preparation of Levamisole

Levamisole (50 mg/ml) was used as a positive control for each series of experiments. PBS buffer was used as the solvent to prepare the target concentration of levamisole.

Isolation of Parasites

A number of infected abomasums were obtained from the Kerman slaughterhouse. First, the connections of the abomasum to the duodenum were ligated and cut. Then, the abomasum was opened and its contents were emptied. To separate the parasite, PBS was gently pushed on the inner surface of the abomasum to loosen the attachments of worms. Ethical considerations and appropriate approvals (46805694) were obtained for all procedures involving animal-derived materials. Among the isolated parasites, those with more movement were selected and placed in fresh buffer. After identifying the genus and species, the parasites were exposed to the extract as well as to positive and negative controls.

Running the Test

The intended concentrations of the ethanolic extract (25, 50, and 75 mg/ml) were prepared, and 20 live and motile parasites were exposed to each concentration. Additionally, a positive control was used with levamisole at a concentration of 50 mg/ml, while a negative control was prepared using 20 ml of PBS. After exposure to the extract, the parasites were kept at a temperature of 25-30 °C. The motility of the parasites was evaluated hourly, and their survival or death was recorded. The measured index was the presence or absence of motility in parasites. The assessment was performed for 7 to 10 hours. To ensure whether they were alive or dead, they were sometimes placed in PBS at a higher temperature (about 35–40 °C). If they showed no movement, they were confirmed dead.

Statistical Analysis

Data analysis was performed using the two-way ANOVA test and a linear regression model in Graph Pad Prism 6.0 software. The levamisole group was considered the base group. To assess the significance of mortality risk in these groups compared to the baseline group, a 95% confidence interval and p-value (0.05) were used.

Results

In this study, the anti-parasitic activity of sumac fruit was investigated. To achieve this goal, an ethanolic extract of sumac was prepared in three concentrations: 25, 50, and 75 mg/ml. Furthermore, a concentration of 50 mg/ml of levamisole was used as the positive control, while PBS was used as the negative control. Twenty worms were exposed to each concentration, and evaluation was performed once an hour in terms of the speed (time) of death. Each study was conducted for up to 10 hours, and the results were recorded in the Table 1.

The results can be seen in the following tables and figure. At the end of the first hour, no deaths were observed in either the positive or negative control groups. In the positive control group, after 4 hours from the start of the exposure, all the worms were completely immobilized. In the negative control, several worms were still alive and moving five hours after the start of the study. No deaths occurred during the first hour at a concentration of 25 mg/ml of ethanolic extract. Under all concentrations of the extracts, 5 hours after the start of the study, all the parasites were immotile. As the duration and concentration increased, the extracts had a greater lethal effect on the worms. As the results show, none of the extracts were significantly different from the levamisole group in terms of inducing immobility. However, all of the concentrations showed a significant difference compared to the negative control. The results of 25 mg/ml showed a significant difference compared to the results of 75 mg/ml.

Table 1. The percentage (%) of live worms in different hours of exposure

Groups	Time				
	1	2	3	4	5
Levamisole 50 (mg/ml)	100	67	33	0	0
Sumac ethanolic extract 25 (mg/ml)	100	93	73	40	0
Sumac ethanolic extract 50 (mg/ml)	80	67	46	7	0
Sumac ethanolic extract 75 (mg/ml)	80	34	20	6	0
PBS buffer	100	87	80	73	67

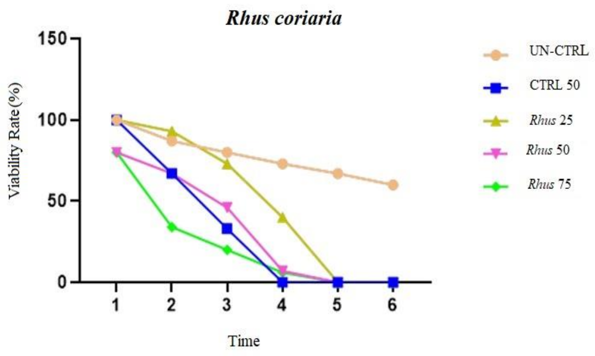

Figure 1. The number of live worms in different hours of exposure to the ethanolic extract of sumac

Discussion

Parasitic infections are among the most significant diseases affecting livestock. In Iran, *Marshallagia marshalli* is the most common abomasal nematode found in domestic and wild ruminants. To reduce the relevant economic and health losses, studies on effective factors should be conducted. In this regard, consideration of the influences of medicinal plants and their active ingredients is particularly essential. Numerous medicinal plants have limited side effects, and their anti-parasitic effects are being investigated.

Today, there are concerns about the formation of drug-resistant pathogens. Gholamian et al. investigated 15 sheep flocks in three geographical regions of Khuzestan province, Iran, and found that 66.6% of the tested flocks were resistant to levamisole. Levamisole resistance has been reported in other parts of the world such as Africa, Australia, and Southeast Asia (14).

Table 2. Results of statistical comparison between different groups

Groups	*p value**	Summary	Significance
Positive and negative control	0.0004	***	Yes
Negative control and concentration 25mg/ml	0.0392	*	Yes
Negative control and concentration of 50mg/ml	0.0004	***	Yes
Negative control and concentration of 75mg/ml	0.0001	****	Yes
positive control and concentration of 25mg/ml	0.2805	NS	No
positive control and concentration of 50mg/ml	0.9999	NS	No
positive control and concentration of 75mg/ml	0.7737	NS	No
Concentration of 25mg/ml and 50mg/ml	0.2805	NS	No
Concentration of 25mg/ml and 75mg/ml	0.0320	*	Yes
Concentration of 50mg/ml and 75mg/ml	0.7737	NS	No

*$p < 0.05$ was notify as significant

Sumac (*Rhus coriaria*) is a medicinal plant with considerable therapeutic properties. This plant mostly grows in subtropical and temperate regions (13). The current research focuses on the effect of sumac on the Marshallagia parasite. Several researchers have studied the anti-parasitic effects of plants on nematodes. Iqbal et al. investigated the anti-parasitic effect of aqueous and methanolic extracts of the *Artemisia breifolia* plant in both laboratory and field environments on sheep infected with the parasite *Haemonchus contortus*. The concentration of both types of extracts used in the laboratory was 25 mg/ml. Experiments conducted in the laboratory showed the effectiveness of both aqueous and methanolic extracts of the mentioned plant on *Haemonchus contortus*. However, the methanolic extract had a stronger effect than the aqueous extract, and the effects of the extracts were less pronounced compared to levamisole, which was used as a control (6). The results of this study confirm the importance of evaluating solvent types as a key factor in optimizing the efficacy of plant-based extracts.

Min et al. determined the effect of tannin in reducing gastrointestinal parasites in rabbits. This study demonstrated that tannins act as bioactive compounds capable of disrupting the parasites' digestive processes by binding with proteins in the gut. Host and colleagues further expanded this by examining the effect of condensed tannins on reducing gastrointestinal nematodes in goats. Their findings indicate that condensed tannins can significantly interfere with the lifecycle of parasites. Additionally, Ramirez et al. investigated the impact of condensed tannin on worm loads of *Haemonchus contortus*, Nematodirus, and Trichostrongylus. Their findings highlighted a significant reduction in worm load, reinforcing the potential of phenolic compounds like tannins as natural anthelmintics (14).

Sumac fruit can act as a rich source of antioxidants due to its phenolic compounds, such as tannins, flavonols, and anthocyanins [15]. Mashhouf investigated the methanolic, ethanolic, and aqueous extracts of *Artemisia santolina* on *Parabronema scriabini*. This study observed that both methanolic and ethanolic extracts were substantially more effective than the aqueous counterpart, reaffirming the greater solubility of active compounds in alcohol-based solvents (16).

Iqbal et al. investigated the anti-parasitic effect of aqueous and methanolic extracts of the tobacco plant on *Haemonchus contortus* in both laboratory and field environments. At a concentration of 25 mg/ml, the aqueous extract killed 70% of parasites after 6 hours, whereas levamisole, used as the control, exhibited complete parasite mortality within the same timeframe. Although less effective than levamisole, this study underlined the potential of plant-based treatments as alternative or supplementary approaches to combating parasitic infections (17).

Tonk et al. evaluated the anti-parasitic effects of *Artemisia annua* on Anopheles *l*arvae. The study found that a concentration of 200 ppm resulted in 100% mortality after 48 hours, showcasing the larvicidal potential of *Artemisia annua* (18). Similarly, Hosseini et al. investigated the effects of thyme, cumin, and geranium plant extracts on different growth stages of *Haemonchus contortus*. Of the three plant extracts, thyme extract proved to be the most effective against the egg and larval stages. However, the adult stage of the parasite was less susceptible to the treatments (19).

Meri-Maghelin et al. investigated the effect of *Cucurbita moschata* plant extract on *Haemonchus contortus*. The dichloromethane extract reduced worm motility after 24 hours. However, its efficacy remained lower than that of levamisole, reinforcing the challenge of achieving synthetic drug-like potency with natural alternatives (20). Lon et al. studied aqueous and methanolic extracts of *Euphorbia helioscopia* on *Haemonchus contortus* in laboratory conditions. The highest mortality rate was observed with methanolic extracts at a concentration of 50 mg/ml, with complete death occurring in the eighth hour. By comparison, levamisole induced total mortality within 4 hours. Despite these differences, the study highlighted the importance of exploring plant-based solutions for nematode management (21).

This study focuses on a significant gap in research by specifically evaluating *Marshallagia marshalli*, a nematode prevalent in ruminants, which has been understudied compared to parasites like *Haemonchus contortus*. It uniquely explores the anti-parasitic properties of *Rhus coriaria*, sumac, a plant with limited prior investigation in this context. By comparing sumac extracts directly to levamisole, a widely used synthetic drug, the study demonstrates the potential of sumac as a natural alternative with comparable efficacy. Unlike many other plant-based studies that primarily target eggs or larvae, this research emphasizes sumac's effectiveness against adult parasites. Furthermore, it highlights the rapid action of sumac extracts, achieving complete parasite mortality within five hours, a performance on par with levamisole. This work provides critical insights into natural anthelmintic solutions to address drug resistance challenges in livestock management.

Conclusion

In our study, sumac extracts caused the death of all *Marshallagia marshalli* parasites within five hours, comparable to levamisole. These findings, combined with its phenolic-rich profile, underscore the significant potential of sumac as a sustainable and effective natural anthelmintic. This study has limitations, including its in vitro nature, which may not fully reflect in vivo conditions, and potential variability in plant extract composition. Future researches should validate these findings through in vivo studies, isolate active compounds, and standardize extraction processes to enhance reproducibility and practical application.

Acknowledgements

The authors wish to express their sincere gratitude to the Faculty of Veterinary Medicine at Shahid Bahonar University of Kerman for their generous financial support, which was instrumental in facilitating this research.

Author Contributions

Soheila Fotoohi: Designed, report and wrote the manuscript, **Elham Mottaghi:** Laboratory evaluation, **Fatemeh Nouri:** helped in writing manuscript, **Saeid Reza Nourollahi Fard:** Conceptualization and supervision, **Mehran Mesgari Abbasi:** Laboratory evaluation.

Data Availability

All data of current study are included in this published article.

Ethical Approval

Ethical considerations and appropriate approvals (46805694) were obtained for all procedures involving animal-derived materials.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Consent for Publication

Not applicable.

Funding

The authors received financial support for the research, from Shahid Bahonar University of Kerman, Kerman, Iran

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