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Original Article

Acaricidal Activity of *Colchicum autumnale* (autumn crocus) Extract against *Hyalomma* spp. *In vitro*

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Abstract

Hyalomma spp. is responsible for the transmission of protozoan, bacterial, rickettsial, and viral diseases and causes huge economic loss to the livestock industry. Recently, there is a wide number of promising attempts to evaluate and use herbal preparations for ticks control. This study aimed to evaluate the acaricidal activity of aqueous and ethanol extracts of *Colchicum autumnale* (*C. autumnale*) rhizome and leaf against the *Hyalomma* spp. *in vitro*. The acaricidal activities of the *Colchicum* leaf aqueous (CLA), *Colchicum* leaf ethanolic (CLE), *Colchicum* rhizome aqueous (CRA), and *Colchicum* rhizome ethanolic (CRE) extracts were evaluated at concentrations of 50, 100, and 150 mg/ml and controls (distilled water and Cypermethrin) following 0.25, 0.5, 0.75, and 1 h of exposure. It is worth mentioning that the spraying method was used in these experiments. Data were analyzed through GraphPad Prism 5 software. In addition, the chemical composition of aqueous leaf extract was analyzed using gas chromatography-mass spectrometry (GC–MS). The carbamodithioic acid (30.04%) was the major chemical constituent identified. Based on the results, CLA, CLE, CRA, and CRE extracts had an acaricidal effect; however, this effect was more potent in CLE. The CLE extract showed a 100% mortality rate at 50, 100, and 150 mg/ml concentrations and 1 h of exposure. The effectiveness of CRA on the *Hyalomma* spp. was very low. The median lethal concentration (LC50) values were obtained at 100 mg/ml. The results indicated that *C. autumnale* leaves contained potent acaricidal ingredients and might provide new acaricidal compounds for the effective control of *Hyalomma* spp. However, further studies are required to evaluate the efficacy of *C. autumnale in vivo.*

Keywords: Acaricide, *Colchicum autumnale*, *Hyalomma* spp.

Activité Acaricide de L'extrait de *Colchicum autumnale* (Crocus d'automne) contre *Hyalomma spp. In vitro*

Résumé: *Hyalomma spp.* est responsable de la transmission de maladies protozoaires, bactériennes, rickettsiales et virales et cause d'énormes pertes économiques à l'industrie de l'élevage. Récemment, il y a eu un grand nombre de tentatives prometteuses pour évaluer et utiliser des préparations à base de plantes pour lutter contre les tiques. Cette étude visait à évaluer l'activité acaricide d'extraits aqueux et éthanoliques de rhizome et de feuilles de *Colchicum autumnale* (***C. autumnale***) contre les *Hyalomma spp In vitro*. Les activités acaricides des extraits aqueux de feuille de *Colchicum* (CLA), éthanoliques de feuille de *Colchicum* (CLE), aqueux de rhizome de *Colchicum* (CRA) et éthanoliques de rhizome de *Colchicum* (CRE) ont été évaluées à des concentrations de 50, 100 et 150 mg/ml et témoins (eau distillée et cyperméthrine) après 0.25, 0.5, 0.75 et 1 h d'exposition. Il convient de mentionner que la méthode de pulvérisation a été utilisée dans ces expériences. Les données ont été analysées via le logiciel GraphPad Prism 5. De plus, la composition chimique de l'extrait aqueux de feuilles a été analysée par chromatographie en phase gazeuse-spectrométrie de masse (GC-MS). L'acide carbamodithioïque (30.04%) était le principal constituant chimique identifié. Sur la base des résultats, les extraits CLA, CLE, CRA

et CRE avaient un effet acaricide; cependant, cet effet était plus puissant dans CLE. L'extrait CLE a montré un taux de mortalité de 100% à des concentrations de 50, 100 et 150 mg/ml et 1 h d'exposition. L'efficacité du CRA sur *Hyalomma spp.* était très faible. Les valeurs de concentration létale médiane (CL50) ont été obtenues à 100 mg/ml. Les résultats ont indiqué que les feuilles de *C. autumnale* contenaient de puissants ingrédients acaricides et pourraient fournir de nouveaux composés acaricides pour la lutte efficace contre *Hyalomma spp*. Cependant, d'autres études sont nécessaires pour évaluer l'efficacité de *C. autumnale in vivo*.
Mots-clés: Acaricide, *Colchicum autumnale, Hyalomma spp.*

1. Introduction

Animal ticks are important hematophagous ectoparasites of vertebrate animals that cause huge economic loss through anorexia, anemia, toxicosis, general stress, negative effects on productivity and the quality of animal's products, depression of immune function, damage to hides, transmission of protozoan, bacterial, rickettsial, and viral pathogens, the rise of treatment costs, as well as mortality in livestock (de la Fuente et al., 2008; Ghosh et al., 2011). *Hyalomma* spp., is a key vector of tick-borne protozoan diseases, such as theilerioses and babesiosis as well as rickettsial diseases, such as anaplasmosis in Iran and many other tropical and subtropical regions. This species attacks cattle and sheep and can cause great economic loss to such livestock (Dagleish et al., 2007).

The use of pesticides has led to an increase in food production. However, due to the fact that pests usually develop rapid resistance to the pesticides in target species, and regarding the toxicity and adverse effects of these agents on human health and their environmental hazards, it is necessary to search continuously for eco-friendly available pesticides (Al-Rajhy et al., 2003). The so-called "green pesticides" are currently claimed to be useful for the control of ectoparasites (Benelli, 2015). Recently, there is a global trend to evaluate and present new safe and effective herbal plants as an alternative to old chemical pesticides due to such features as low cost; availability; low environmental contamination; low side effects, toxicity, and resistance. It should be noted that medicinal plants are both cheap and effective for the control of cattle ticks (Babar et al., 2012). Chemical acaricides, such as Ivermectin and Cypermethrin have been widely used to treat and control ticks in veterinary clinics with relatively good effectiveness.

Colchicum autumnale, commonly known as the autumn crocus, wild saffron, and naked lady, contains alkaloid Colchicum that is an antimitotic agent and blocks the mitosis by preventing DNA synthesis and tubulin polymerization (Brvar et al., 2004). *Colchicum* has been responsible for numerous intoxications and deaths and is used in the management of acute gouty arthritis. *Colchicum* tablet overdose is the most common cause of *Colchicum* poisoning (Mullins et al., 2000). There was a report of toxicity in cattle after some rural farmers in Ardebil Province, northwest of Iran, used *C. autumnale* to control ticks. Obviously, these animals should not be used as a food source (Kupper et al., 2010). To the best of our knowledge, the acaricidal activity of C. *autumnale* has not been studied to this date. In this study, we evaluated the acaricidal activity of aqueous and ethanol extracts of *C. autumnale* rhizome and leaf against *Hyalomma* spp. through *in vitro* assays.

2. Material and Methods

2.1. Plant Material

C. autumnale was collected from Ardebil Province, northwest Iran, in October 2018. The plant was identified and authenticated by the Medical Pharmaceutics Sciences Department of Faculty of Pharmacy in Tabriz University of Medical Sciences, Tabriz, Iran. A voucher specimen with accession number ZSY112 was submitted to the Herbarium of Medical Pharmaceutics Sciences department of the

Pharmacy Faculty in Tabriz University of Medical Sciences, Tabriz, Iran.

2.2. Preparation for Extraction

The leaves and rhizomes of *C. autumnale* were left to dry under laboratory conditions for two weeks. The dry roots and leaves of the plant were powdered using mortar and pestle and screened using an 80-mesh screen. The aqueous extract of the leaves and rhizome parts of *C. autumnale* was prepared as follows. Initially, 100 gr powder of herbal *C. autumnale* was macerated with 300 ml water three times (each time for 24 h). Subsequently, the flask was agitated for frequent mixing over a period of 4 h. Afterward, the mixtures were completely air-dried in an incubator at 37 °C. Eventually, the extract was scrapped off, transferred to an air-tight container after determination of yield percentage, and was stored in a freezer at -20 °C until the subsequent use.

For the preparation of ethanol extracts of *C. autumnale*, 100 gr herbal *C. autumnale* powder was soaked in 300 ml ethanol for 24 h and the ethanol extract of *C. autumnale* was obtained after they were filtered, concentrated, and evaporated. The working concentrations (50, 100, and 150 mg/ml) of aqueous and ethanolic extracts were prepared by dissolving the required quantity of dried extracts in distilled water or ethanol, respectively to test their acaricidal potential against *Hyalomma* spp.

2.3. Collection of Ticks

Female ticks were randomly collected from naturally infected sheep and cattle. Initially, ticks were collected and placed in Petri dishes. Afterward, all the ticks with the same weight range were selected. Petri dishes were examined under a stereomicroscope and the species of the tick were determined in the laboratory.

2.4. Acaricidal Activity *In vitro*

In an *in vitro* experiment, the anti-tick activity of four extracts (rhizome aqueous, rhizome ethanol, leaf aqueous, and leaf ethanol) was studied at 50, 100, and 150 mg/ml doses. Subsequently, the acaricidal activity of the four extracts with higher mortality was studied. All extracts were diluted in distilled water to adjust different concentrations. One ml of each extract was added to the plate, separately, and the extracts were absorbed with filter papers. Afterward, ten adult ticks (in the same weight range) were collected from the naturally infected animals and were placed in each Petri dish. Subsequently, different concentrations of the plant were sprayed directly on the ticks and they were examined every 0.25, 0.5, 0.75, and one hour. Three replicates were performed for each extract concentration (Kirkwood, 1963). Similarly, Cypermethrin (Cypermethrin 10%, Hacker, Iran) at a similar concentration and distilled water were used as the positive and negative controls.

2.5. Statistical Analysis

The data were analyzed using GraphPad Prism software program (version 5) through a two-way ANOVA, Student's two-tailed t-test for the comparison between test and control and presented as a mean ± SD. Eventually, the median lethal concentration (LC50) was calculated.

3. Results

Based on the results, all extracts of *C. autumnale* had anti-tick effects against *Hyalomma* spp. at all test times and concentrations. It should be noted that CLE extract had the highest anti-tick effect. The anti-tick effect of ethanol extract of *C. autumnale* was stronger than aqueous extracts. The CRA had a very low effect on the *Hyalomma* spp. The mean mortality rate of ticks after 1 h exposure to leaf ethanol extracts (50, 100, and 150 mg/ml) was 100%. The mortality rate of ticks after exposure to different concentrations of the *C. autumnale* extracts at various exposure times are presented in Table 1 and Figures 1, Figure 2 and Figure 3.

Gas-chromatography/mass spectrometry (GC-MS) investigation showed that Carbamodithioic acid (30.04%) as the main ingredient of *C. autumnale* has been isolated from this plant. The results of the High-Performance Liquid Chromatography (HPLC) investigation are presented in Table 2 and Table 3. The statistical results demonstrated that *C. autumnale* plant acted as a good pesticide against *Hyalomma* spp. and was recommended to be used as an acaricidal plant.

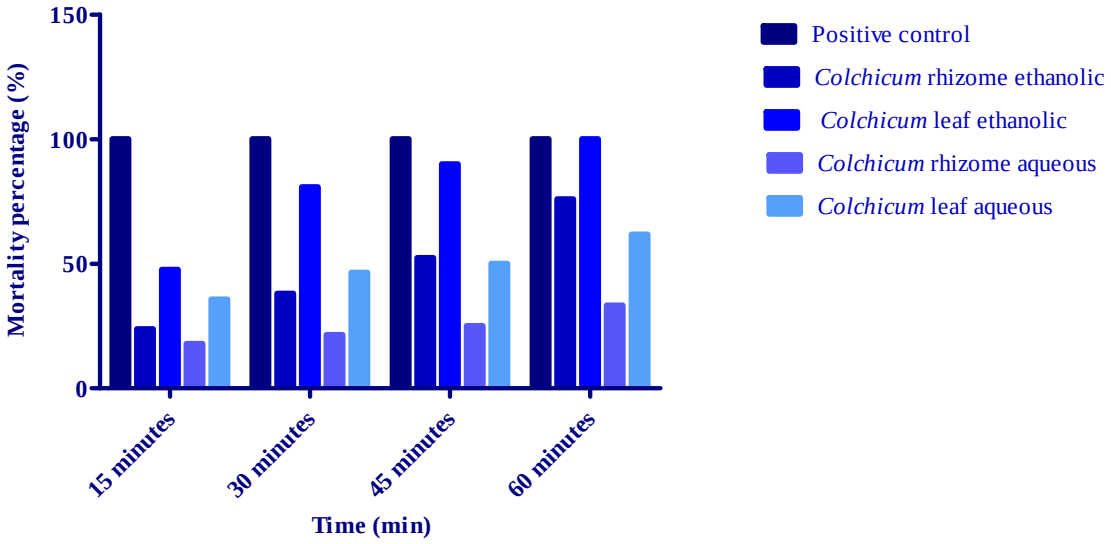

Figure 1. Acaricidal effects of 50 mg/ml concentration of *C. autumnale* in various exposure times

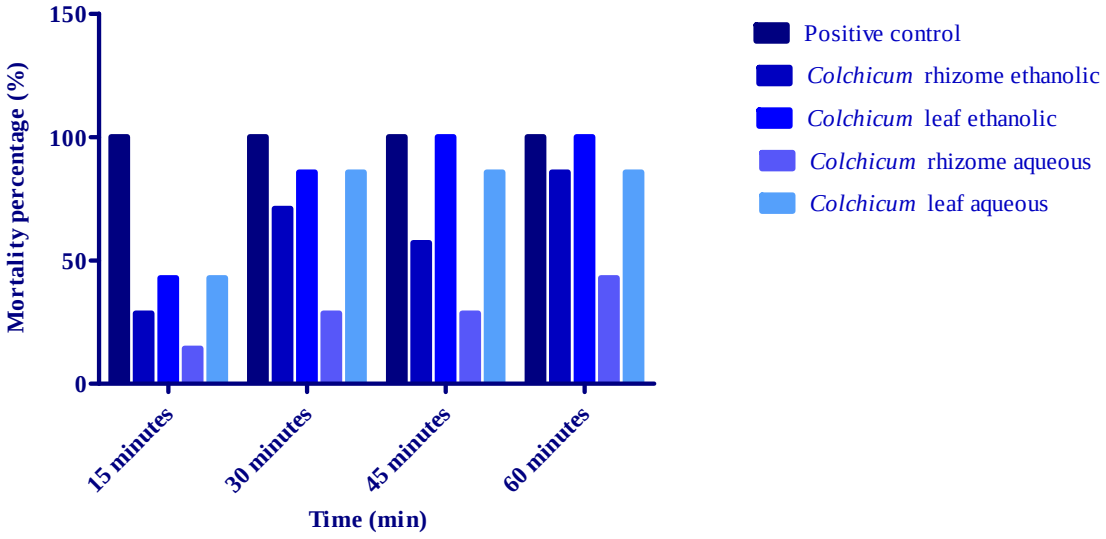

Figure 2. Acaricidal effects of 100 mg/ml concentration of *C. autumnale* in various exposure times

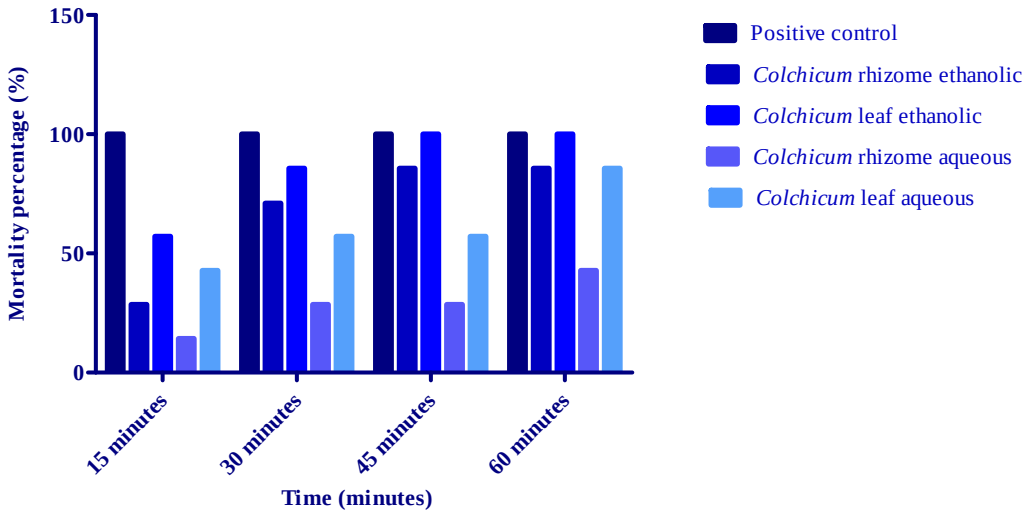

Figure 3. Acaricidal effects of 150 mg/ml concentration of *C. autumnale* in various exposure times

Table 1. The acaricidal activity of the extracts of the *C. autumnale* against *Hyalomma* spp. *In vitro*

	Times	**Positive control**	***Colchicum* rhizome ethanolic**	***Colchicum* leaf ethanolic**	***Colchicum* rhizome aqueous**	***Colchicum* leaf aqueous**	**Negative control**
50 mg/ml	15 min	100±0.0	23.81±4.76	47.61±4.76	17.85±3.57	35.71±4.12	0.0±0.0
	30 min	100±0.0	38.09±4.76	80.81±4.89	21.43±4.12	46.42±3.57	0.0±0.0
	45 min	100±0.0	52.38±4.76	90±3.57	25±3.57	50.00±4.12	0.0±0.0
	60 min	100±0.0	75.92±4.89	100±0.0	33.33±4.76	61.77±4.62	0.0±0.0
100 mg/ml	15 min	100±0.0	33.33±4.76	42.85±8.24	19.04±4.76	47.61±4.76	0.0±0.0
	30 min	100±0.0	50.00±4.12	80.81±4.89	25.00±3.57	80.81±4.89	0.0±0.0
	45 min	100±0.0	66.39±4.62	95.24±4.76	38.09±4.76	82.04±3.67	0.0±0.0
	60 min	100±0.0	80.81±4.89	100±0.0	47.61±4.76	89.28±3.57	0.0±0.0
150 mg/ml	15 min	100±0.0	33.33±4.76	57.04±5.75	42.85±3.53	42.42±3.53	0.0±0.0
	30 min	100±0.0	66.39±4.62	80.81±4.89	57.14±4.62	57.61±4.62	0.0±0.0
	45 min	100±0.0	80.81±4.89	92±3.57	61.77±4.62	57.61±4.62	0.0±0.0
	60 min	100±0.0	90.47±4.76	100±0.0	67.75±6.82	89.71±3.57	0.0±0.0

Table 2. HPLC results of ingredients and percent (%) of *C. autumnale* aqueous extracts

Percent (%)	Ingredients	Percent (%)	Ingredients
1.07	Heneicosane	0.80	Chloroform, Methan
0.59	1-Bromoeicosane	0.56	Trichlormethan
0.76	Valeric acid	0.54	Ethan,1,2,2-Trichro-1
0.26	1,3-Dioxolane	0.54	Methane,Bromodichloro-(CAS)
0.83	Malonic acid	0.29	Eicosane, 3-Cyelohexyl
0.83	O-Nitroacetophenone Oxim	0.29	Permetrinic acid methylamide
0.58	8-d-Thiocamphor	0.84	Acetate
0.58	Triacontane	0.40	9-Tricosene
1.16	Dotriacontane	0.40	Octadecan
1.87	1-Bromo-11-Iodoundecan	0.40	Nonadecane
0.18	Bemegride Methyl derivative	0.29	4-Nitrobenzoic acid
0.18	2-Methyl-7-Phenylindole	0.34	P-Menthan-3-one,Semicarbazone
1.28	N-Methyl-2 Iido- Pyrrole	0.47	11,15-Dimethyl Heptatriacontane
1.13	Methoxyacetic acid	0.47	Cyclolexane

Table 3. HPLC results of ingredients and percent (%) of *C. autumnale* ethanolic extracts

Percent (%)	Ingredients	Percent (%)	Ingredients
0.89	1-Bromo-11-iodoundecane	25.2	Heptaflourobutyrice anhydride
0.89	Valeric acid, 2, 6 Dimethylnon	25.2	Pentaflouropropionic anhydride
3.05	3,5-Dimethoxyacetophenon	30.04	H-Benzo[4,5]furo[3,2-f]indole …
1.93	Nonacosane	30.05	Carbamodithioic acid
1.93	Triacontane	2.67	Pyridine, Petaflouoro-(CAS)
1.04	Hentriacontane	2.67	2-thiophenecarboxylice acid
0.24	Cyclododecanone	2.67	p-Menthan-3-one, Semicarbazone, (1R,4R)-; Isomenth
1.15	Phthalic acid	0.41	Dodecane(CAS), n-Dodecan
1.17	4,5.alpha.-Epoxy-3-methoxy-17	0.41	Tridecan
1.89	Quinoline	1.04	1,2-Benzisothiazole
1.57	Glycine	1.18	Thiono[2,3-c]pyridine
1.39	2-[3-(4-tert-Butyl-phenoxy)	3.89	Thiocyanic acid
0.66	1,2-Bis(trimethylsilyl)	2.58	4,5.alpha.-Epoxy-3-methoxy
1.57	N-Ecosan	1.75	n-Pentacosane

4. Discussion

Herbal therapies are natural, safe, environment friendly, and cheap products that develop resistance slowly and have limited side effects (Olivo et al., 2009). The tick infestation limits the productivity of animals. The number of laboratory studies on natural products that can be used for the control of ticks as a replacement for synthetic compounds has grown in recent years. The antiparasitic properties of many ethnoveterinary plant extracts and essential oils have been tested with success (Athanasiadou et al., 2007; Masood et al., 2013; Abbas et al., 2014). Moreover, *in vitro* assays indicated that more than 200 plant species from different countries around the world have tick-

repellent or acaricidal properties (Adenubi et al., 2016). Several studies have reported remarkable acaricidal properties of natural plants *in vitro* and/or *in vivo*, such as *Azadirachta indica, Syzygium aromaticum, Stemona sessilifolia, Eupatorium adenophorum, Gynandropsis gynandra, Lavendula augustifolia, Pelargonium roseum, Cymbopogon* spp. *Cedrus deodara, Pongamia glabra, Copaifera reticulata* and *Jatropha curcas* (Borges et al., 2003; Du et al., 2008; Ribeiro et al., 2008; Magadum et al., 2009; Pirali-Kheirabadi and Teixeira da Silva, 2010; Ghosh et al., 2011; Ravindran et al., 2011; Nong et al., 2012; Politi et al., 2012; Godara et al., 2014; Singh et al., 2014; Adenubi et al., 2016).

Local people in Ardebil Province, northwest of Iran, used *C. autumnale* to control ticks in animals. In this paper, the acaricidal activities of *C. autumnale* against *Hyalomma* spp. *in vitro* were tested. The results demonstrated that all extracts of *C. autumnale* showed anti-tick effects against *Hyalomma* spp. at all test times and concentrations. Moreover, ethanol extract showed better acaricidal activity, compared to aqueous extract. The mean mortality rate with 60 min exposure to leaf ethanol extracts (50, 100 and, 150 mg/ml) was 100%.

Al-Rajhy et al. (2003) investigated the efficacy of the cardiac glycoside, digitoxin, from *Digitalis purpurea L.* (Scrophulariaceae), a cardiac glycoside (cardenolide) extract from *Calotropis procera* (Ait) R. Br. (Asclepiadaceae), and azadirachtin and neem oil from *Azadirachta indica A. Juss* (Meliaceae) for their effects against larvae and adult stages of the camel tick, known as *Hyalomma dromedarii* Koch (Acari: Ixodidae). The contact LC50 values of the first three materials against adult ticks were estimated at 4.08, 9.63, and >40.7 mg/cm^2, respectively, whereas the dipping LC50 values of the three materials were 409.9, 1096, >5000, and >5000 mg/L, respectively. Contact and dipping LC50 values of the extract and azadirachtin against larvae were 6.16, >20.3 mg/cm^2 587.7, and >2500 mg/L, respectively. *Azadirachtin* had no effects on egg production or feeding of adults up to 5000 mg/L; however, at 2500 mg/L, it caused a significant reduction in feeding activity of larvae, which prolonged the period for moulting to the nymphal stage, and caused a 60% reduction in moulting process. Results of the two cardiac glycoside materials were compared with those of several commercial acaricides. The risks and benefits associated with the use of cardiac glycosides were considered**.** The dipping time in this study was 30 seconds (Al-Rajhy et al., 2003).

In this paper, the preliminary tests demonstrated that *C. autumnale* extract has significant acaricidal activity against *Hyalomma* spp. *in vitro*, and ethanol extract was found to be the active fraction of *C. autumnale*. Further studies need to be conducted in an *in vivo* condition to develop new potential medicines for the control of ticks in livestock.

Authors' Contribution

Study concept and design: R. N.
Acquisition of data: Armin Sh.
Analysis and interpretation of data: M. H.
Drafting of the manuscript: R. N.
Critical revision of the manuscript for important intellectual content: Arman Sh.
Statistical analysis: R. N.
Administrative, technical, and material support: R. N.

Ethics

This work has been performed under laboratory conditions; therefore, ethical committee approval was not needed.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study

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