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RESEARCH ARTICLE

In Vitro Anthelmintic Activity of Five Different *Artemisia L.* Species Growing in Türkiye

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A B S T R A C T

This study aims to determinate *in vitro* anthelmintic activity of the plant extracts from the aerial part of five different *Artemisia* L. species (*A. absinthium* L., *A. abrotanum* L., *A. annua* L., *A. incana (*L*.) Druce*, *A. tournefortiana* Rchb.) growing in Türkiye. Depending on the ethnobotanical usage, the aqueous plant extracts were prepared by decoction and maceration methods. The anthelmintic activity of the plant extracts on *Haemonchus (H.) contortus* eggs and larvae was assessed using *in vitro* test methods. The extracts were prepared in five concentrations (50; 25; 12.5; 6,25; 3.125 and 1.5625 mg/mL) by using PBS for egg hatch and larval motility inhibition assay. In comparison to the negative control group, the extracts had significant anthelmintic effect on *H. contortus* eggs and larvae ($p<0.05$). In the egg hatch assay, all plants were found to be fully effective in the concentration range studied. In the analysis of larval motility, the activity was correlated with the quantities of the plant extracts. When the concentration-activity values of the plants were examined for both extraction methods, the highest activity was in *A. annua* and the lowest activity was observed in *A. tournefortiana*. Due to the development of resistance to anthelmintic drugs, natural sources such as *Artemisia* species can be considered as an alternative for the treatment of nematodes.

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INTRODUCTION

Gastrointestinal (GI) nematodes are common and important problems in pasture animals worldwide and they lead to decrease in animal health and productivity. Nematodes that infect the digestive system can result in a variety of clinical consequences, including long-term and irreversible morbidity in both humans and animals (Vande Velde *et al*., 2018; Zajíčková *et al*., 2020). *Haemonchus (H.) contortus*, a prevalent parasite affecting small ruminants globally. It is widely acknowledged as an exemplary pathogen and serves as an important research subject for drug development due to its suitability for experimental studies (Doyle *et al.*, 2020).

Anthelmintic drugs are mainly used for treatment of GI helminths, but alternative approaches are needed to address the emergence of anthelmintic resistance (Erez and Kozan, 2018; Verma *et al*., 2018). Natural resources can be used to control these parasitic diseases in addition to contemporary anthelmintic medications. Different cultures have long employed various medicinal plants as anthelmintics (Kozan *et al.*, 2016). On the other hand, the restriction on the use of antiparasitic medications in livestock gives researchers and livestock experts the chance to create substitute feed additives that are secure for both livestock and consumers of foods derived from animals. However, organic producers favor a non-pharmaceutical strategy for worm management. Consequently, phytotherapy may be a suitable substitute (Van Krimpen *et al*., 2009; Ramdani *et al*., 2023).

Small plants and shrubs belonging to the Asteraceae genus *Artemisia L.* can be found in Asia, North America and Europe. The genus *Artemisia* is part of the Anthemideae tribe, which comprises over 500 species (Abad *et al*., 2012; Taleghani *et al*., 2020). They are frequently employed in the treatment of various illnesses, including hepatitis, inflammation, malaria, viral, bacterial and fungal infections (Abad *et al*., 2012). *Artemisia* species have attracted considerable attention in many countries, particularly due to the presence of artemisinin, the primary

sesquiterpene found in *Artemisia annua*. Artemisinin has been recommended by the World Health Organization as an antimalarial drug for treating quinine-resistant malaria (Ferreira, 2009). Phytochemical studies have revealed that *Artemisia* species predominantly contain coumarins, flavonoids, terpenoids, sterols, acetylene compounds and have great potential to yield essential oils (Bora and Sharma, 2011; Zeb *et al*., 2019).

Ethnobotanical studies conducted in Türkiye have shown that *Artemisia* species have been traditionally used for various purposes such as anthelmintic, antimalarial, antipyretic, antihypertensive, appetizing, wound healing, stomachic, diuretic, tonic, and sedative effects. They have also been used in the treatment of diseases such as diabetes, asthma, ulcer, and cold (Altundag and Ozturk, 2011; Paksoy *et al*., 2016; Güneş *et al*., 2017). However, a literature review indicates that no scientific study has been published on the anthelmintic activity of five different *Artemisia* species that growing in Türkiye, namely *A. absinthium L., A. abrotanum L., A. annua, A. incana (L.) Druce, and A. tournefortiana* Rchb. Therefore, the objective of this research is to assess the *in vitro* anthelmintic activity of these species towards *H. contortus*, aiming to provide scientific validation for their traditional use in ethnobotany.

MATERIALS AND METHODS

Plant material: Names of collected species, the dates and localities of their collection and the herbarium numbers are given in Table 1.

Preparation of the plant extracts

Decoction with distilled water: For each plant, 15 g of shade-dried and powdered herb was boiled in 150 mL of distilled water for three minutes (Paksoy *et al*., 2016). After boiling, the mixture was filtered. The obtained extracts were then evaporated using a lyophilizer (Christ Gamma 2-16 LSC) at -40°C for one week. The yields of each extract were as follows: 7.46, 7.46, 8.66, 8.06 and 12.06% for *A. abrotanum*, *A. absinthium*, *A. annua*, *A. incana* and *A. Tournefortiana*, respectively. The resulting residues were stored at -20°C until they were used.

Maceration with distilled water: For each plant, 15 g of shade-dried and powdered herb was macerated in 150 mL of distilled water at room temperature for 24 hours followed by filtering through a filter paper (Sekiou *et al*., 2019). Throughout the process, the samples were stored in the refrigerator at 4°C. After filtration, the extracts were evaporated using a lyophilizer (Christ Gamma 2-16 LSC) at -40°C for one week. The yields of each extract were as follows: 12.2% for *A. abrotanum*, 9.93% for *A. absinthium*, 8% for *A. annua*, 10.26% for *A. incana*, and 10.73% for *A. tournefortiana*. The obtained residues were stored at -20°C until they were used.

***In vitro* biological activity assay:** For the purpose of determining the anthelmintic action of the extracts, both the egg hatch assay (EHA) and larval motility inhibition assays were employed.

Egg hatch assay: Fecal samples were collected from naturally infected commercial sheep herd in the province of Antalya for extracting *Haemonchus* eggs (Coles *et al*., 1992). The eggs were prepared at a concentration of 100-200 eggs/mL following the McMaster procedure. The supernatant was collected after the suspension was centrifuged (Nüve NF 815) for 5 minutes at 1500 rpm. A 48-well microtiter plate was filled with approximately 100 eggs in 200 µL of distilled water. Each test well was then supplemented with 200 µL of plant extract at doses of 50, 25, 12.5, 6, 3, and 1.5625 µL, resulting in a total volume of 400 µL per well. Similarly, 200 µL of 99.8% PBS was used as a negative control, and 200 µL of 99.8% albendazole at a concentration of 0.25 mg/mL was used as a positive control. All plates were incubated at 27°C for 48 hours. To prevent further hatching, a drop of Lugol's iodine solution was added to each well. All unhatched eggs and L_1 larvae in each well were counted using an Olympus CX21 microscope. Finally, the percent inhibition of egg hatching was calculated:

Percent inhibition = 100 (1 − P*test*/P*control*), where P= number of eggs hatched in EHA.

Preparation and maturation of trichostrongylid larvae: One kilogram of feces was collected from a naturally infected sheep flock with *H. contortus* in Antalya Province. In a plastic container, feces were mixed with wood shavings then incubated at 27°C (Nüve ES 110) for 10-11 days to hatch the *H. contortus* eggs and develop into infective L_3. The mixture was transferred to Baermann's apparatus after incubation and left overnight the larvae to migrate. The L_3 stage parasites were extracted from the test tubes attached to the end of the Baermann apparatus, concentrated through centrifugation (5 times at 1000 rpm for 5 minutes), picked up using a micropipette, and washed three times with PBS. The larvae were stored at 8°C for future use (Molan *et al*., 2003).

Larval motility assays: For modified larval motility assay (Kotze *et al*., 2004), distilled water was used to dissolve the dried extracts, along with 5% dimethyl sulfoxide (DMSO). A total of 100 larvae were incubated with plant extracts at concentrations of 50, 25, 12.5, 6.25, 3.125, and 1.5625 mg/mL of PBS (pH 7.2; temperature 15-30°C), as well as with PBS alone. Albendazole was also included as a positive control at various concentrations (50, 25, 12.5, 6.25, 3.125 and 1.5625 g/mL). The mortality was observed at 0, 1, 2, 3, 4, 6, 8, and 24-hour intervals. The percentage of dead larvae was calculated based on criteria such as brightness, straightness and lack of motility in non-moving larvae. Three replicates were performed per concentration of each extract.

Data Analysis: The effect of the extraction methods used in the experiment was evaluated statistically. The Chi-square test, Kruskal Wallis Test, and Mann-Whitney Test were used in data analysis, and $p<0.05$ values were considered significant. 23.0 SPSS program was used for statistical analysis.

RESULTS

In this study, the anthelmintic activities of the aqueous extracts of five different *Artemisia* species (*A. absinthium, A. abrotanum, A. annua, A. incana* and *A. tournefortiana*) prepared by two different extraction methods (maceration and decoction) depending on the concentration were evaluated.

Table 1: Names, collection dates/localities, and the herbarium numbers

Species	Locality and Date of Collection	AEF*	HUEF**
ABR	C5, Bitlis: Güroymak, between Muş and Güroymak, 500 m away from Güroymak district, roadside, streamside, 1290 m, 05.09.2019	28823	21003
ABST	B5, Kars: 1 km from the city center, on the Tuzluca-Kars road, among the rubble piles on the roadside, 1765 m, 10.08.2019	28821	21002
ANN	B1, Sakarya, Akyazı, on the Kuzuluk road, roadside, 13. 10.2019	28820	21001
AINC	C5, Muş: Malazgirt, Between Aktuzla village and Karıcalı village, roadside, slopes, 1560 m, 09.08.2019	28822	21004
ATOR	B5, Kars: 1 km from the city center, on the Tuzluca-Kars road, among the rubble piles on the roadside, 1765 m, 10.08.2019	28824	21005

*AEF: *Ankara University* Faculty of Pharmacy Herbarium: **HUEF: *Hacettepe University* Faculty of Pharmacy Herbarium: ABST: *A. absinthium* L., ABR: *A. abrotanum* L., ANN: *A. annua* L., AINC: *A. incana* (L.) Druce, ATOR: *A. tournefortiana* Rchb

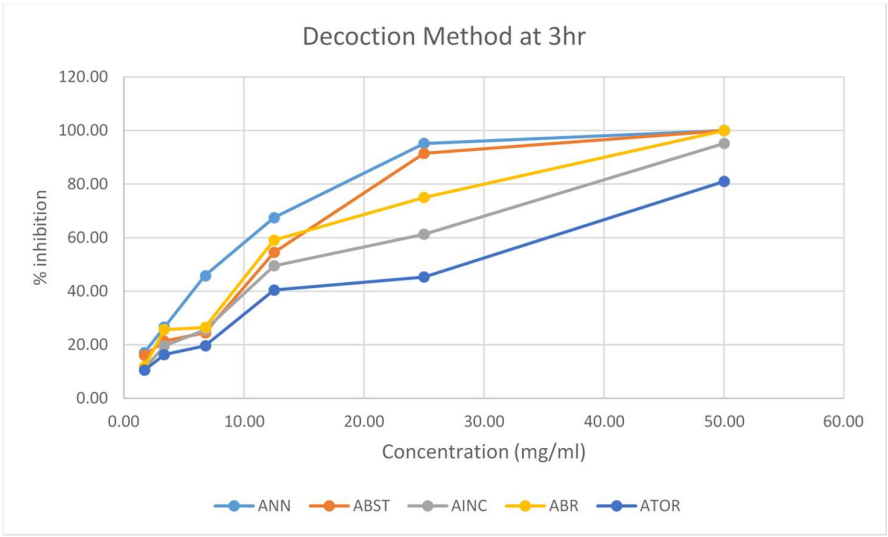

Fig. 1: The nematocidal effect of aqueous extracts of *Artemisia* species prepared by the decoction method against concentration (%) at 3rd hour.

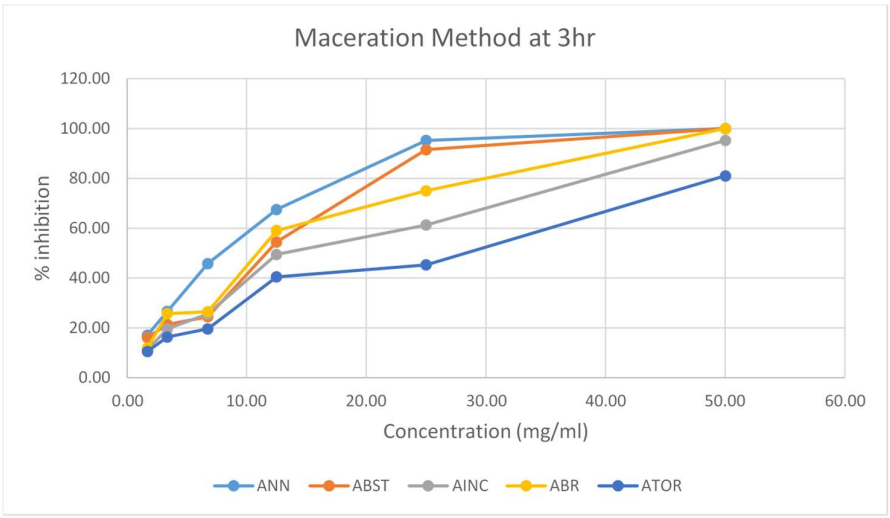

Fig. 2: The nematocidal effect of aqueous extracts of *Artemisia* species prepared by the maceration method against concentration (%) at 3rd hour.

In the egg hatch inhibition assay, all extracts were 100% effective at all studied concentrations (50-1.5625 mg/mL) and at all-time intervals (1-48 hours). Considering the nematocidal activity of the extracts on *H. contortus* larvae, increased activity of all plants was determined depending on the concentration (Fig. 1-4). When the concentration-activity values of the plants were examined for both extraction methods, the highest activity was in *A. annua* (Fig. 1 and 2); the lowest activity was observed in *A. tournefortiana* at 3rd hour. After 24 hours, the LC50 values for the extracts prepared by the decoction method were 5.06 for *A. abrotanum*, 6.037 for *A. annua* 6.78 for *A. incana*, 6.955 for *A. absinthium*, and 10.568 for *A. toruneforiana;* for the extracts prepared by the maceration method were 5.94 for *A. abrotanum*, 5.99 for *A. absinthium*, 10.56 for *A. annua,* 12.80 for *A. incana*, and 13.67 for *A. toruneforiana* (Table 2). When statistical data are evaluated, there is no statistically significant difference in terms of plants since ($p>0.05$) on hourly basis for both extraction methods. There is no statistically significant difference in terms of extracts ($p>0.05$) for all plants (Table 3). This may be due to the small number of studied parameters. When the activities were evaluated on an hourly basis, it was observed that the first extract that reached 100% efficiency (in 2 hours) was the aqueous decoction extract of *A. annua* at a dose of 50 mg/mL.

Table 2: The mortality **(%)** and LC50 (ppm) values at 24 hr of plant extract obtained by different methods on 3rd instar larvae of *H. contortus.*

Extraction method	Plant name	Consantration (mg/mL)	Mortality (%)							Lethal concentration 50 (LC_{50}) for L_3 stages *of Haemonchus contortus*
			1.hr	2.hr	3.hr	4.hr	6.hr	8.hr	24.hr	
Decoction	ANN	50	80	100	100	100	100	100	100	6.037
		25	42,5	90,9	95,2	100	100	100	100	
		12,50	29,3	62,5	67,5	72,5	75	80	88,8	
		6,25	24	41,6	45,8	50	52,7	55,5	58,3	
		3,125	15	21, 60	26, 60	28, 30	31,6	35	40	
		1,5625	9, 75	14,6	17,07	19,51	20,73	24,39	36,40	
	ABST	50	72	92,6	100	100	100	100	100	
		25	36	83,3	91,5	100	100	100	100	6.9554
		12,50	25,7	46,1	54,5	70,5	70,5	80	85,6	
		6,25	20, 40	22, 44	24,48	28, 57	30	38	45,50	
		3,125	10,71	16,07	21,40	25	25	32,14	36,80	
		1,5625	7,14	12,50	16,07	16,07	17,80	21,40	26,80	
	AINC	50	66	88,88	95,2	100	100	100	100	6.7803
		25	28,57	44,44	61,3	76,4	85	88,88	100	
		12,50	25	36,36	49,5	66,8	72,22	75	82,75	
		6,25	18,4	22,6	25,6	31,5	35,8	39,3	44,3	
		3,125	14,4	17,55	19,6	24,4	31,5	34,2	38,75	
		1,5625	10	10	11,11	13,33	13,33	16, 66	18,6	
	ABR	50	48,34	92,4	100	100	100	100	100	
		25	42,8	66,6	75	83,3	87,5	95,8	100	5.0691
		12,50	36,48	52,17	59,09	75	81,81	93,18	100	
		6,25	23,6	24,4	26,5	32,6	36,7	42,85	49,4	
		3,125	17,14	20	25,7	31,4	37,14	37,14	41,8	
		1,5625	6,57	10,52	11,84	15,7	15,7	18,4	21,6	
	ATOR	50	45	72	81	100	100	100	100	8.7556
		25	22,5	37,14	45,3	50	79,82	84,4	88,88	
		12,50	20,68	19,6	40,45	40	65,7	70	75	
		6,25	15,9	17,4	19,6	29,8	31,5	32,8	40,35	
		3,125	12,56	12,35	16,35	21,56	24,4	27,45	33,5	
		1,5625	8,5	9,5	10,5	16,9	10	11,95	15,3	
Maceration	ANN	50	69,75	88,88	100	100	100	100	100	10.5682
		25	70,4	82,5	85,71	85,71	87,4	88,88	90,5	
		12,50	32,2	38,9	42,8	46,3	49,65	60,5	67,5	
		6,25	17,75	20,35	24,5	28,45	38,36	40,8	45,65	
		3,125	11,3	15,45	18	20,4	24,8	27,5	30	
		1,5625	9,6	11,8	13,5	16,5	16,5	17,7	21,3	
	ABST	50	50	68,6	75	88,88	92,8	100	100	5.9972
		25	28,8	42,8	64,5	69,35	78,25	82,5	93,5	
		12,50	23,56	35,6	48,9	57,1	63,6	70	75	
		6,25	18,4	20,8	21,9	30,6	42,3	48,5	61,55	
		3,125	10,56	15,5	20,8	23,6	25,14	29,9	43,4	
		1,5625	8,9	12,3	14,55	15,8	16,3	20,75	25,5	
	AINC	50	52,4	62,55	75	79,45	96,8	100	100	
		25	25,45	39,35	48,6	59,2	68,7	70,44	76,75	12.8039
		12,50	28,56	32,8	42,7	45,76	64,9	68,45	72,9	
		6,25	20,56	22,2	23,15	29,75	33,25	36,8	42,32	
		3,125	12,96	13,35	13,35	16,8	21,55	27,24	30,46	
		1,5625	7,85	8,85	10	11,6	12	14,47	16,85	
	ABR	50	45,75	66,35	67,35	79,72	92,43	100	100	
		25	36,5	52,68	62,5	80	75,6	82,9	96,56	5.9481
		12,50	20, 80	44,4	52,75	66,47	79,76	79,76	90,3	
		6,25	14,8	22,42	24,85	34,5	34,9	42,2	45,7	
		3,125	16	13,65	18,35	30,6	28,7	36,5	36,35	
		1,5625	7, 96	10,25	10,8	15	13,45	18,3	18,85	
	ATOR	50	35	65	78,9	100	100	100	100	13.671
		25	20,7	32,5	41,5	46,5	72,4	81,3	81,9	
		12,50	18,5	18,5	36,8	36,8	60	64,25	69,45	
		6,25	14	14	19	25	27,45	29,45	35,5	
		3,125	12,15	13,65	15,5	19,6	20,35	25	30	
		1,5625	6	8,5	9,7	12,3	12,3	13,6	14,6	
Albendazole		0.25	21.45	30.5	42.6	55.65	55.65	60	60	
PBS		0	0	0	0	0	0	0	0	-

ABST: *A. absinthium* L.. ABR: *A. abrotanum* L.. ANN: *A. annua* L.. AINC: *A. incana* (L.) Druce. ATOR: *A. tournefortiana* Rchb.

DISCUSSION

Anthelmintic medication is the primary technique for controlling GI nematodes. There are only a few anthelmintic medication classes in the market and the majority of livestock parasite species have evolved significant resistance to these drugs. For long-term parasite control, it will be essential to create novel anthelmintics with various mechanisms of action. Plants are a great source of naturally occurring substances that can be utilized as substitutes for dewormers in livestock (Jayawardene *et al*., 2018; Ahmed *et al*., 2023).

In the current *in vitro* study, aqueous extracts of all studied *Artemisia* species (*A. absinthium, A. abrotanum, A. annua, A. incana* and *A. tournefortiana*) were found to be 100% effective on *H. contortus* eggs. In addition, the

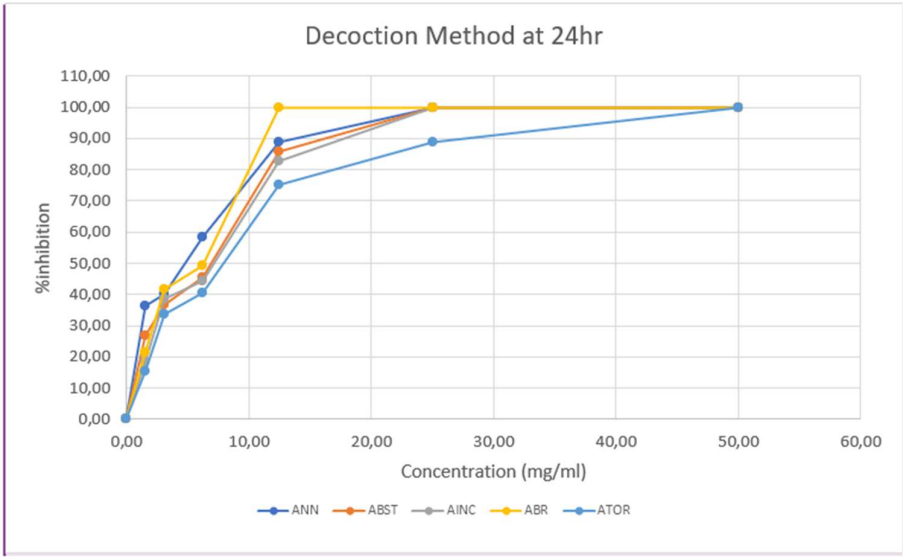

Fig. 3: The nematocidal effect of aqueous extracts of *Artemisia* species prepared by the decoction method against concentration (%) at the 24th hour.

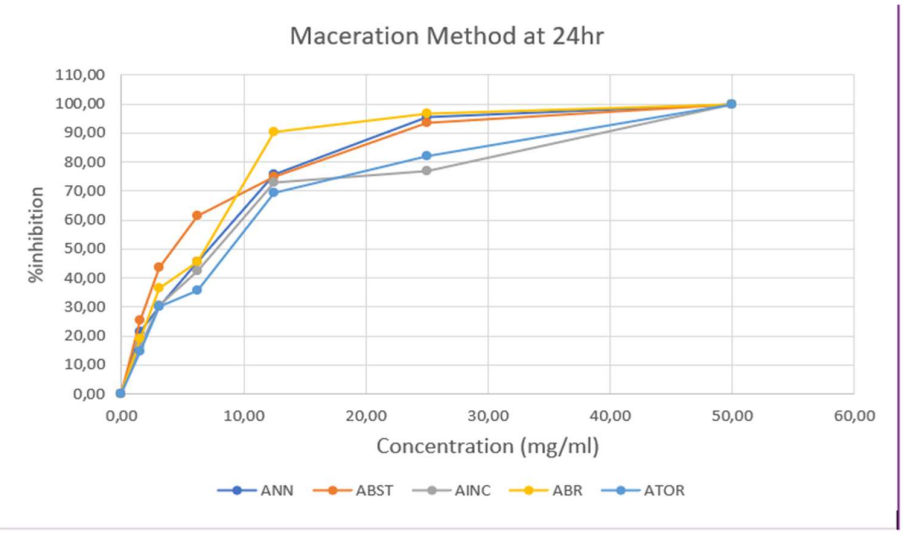

Fig. 4: The nematocidal effect of aqueous extracts of *Artemisia* species prepared by the maceration method against concentration (%) at the 24th hour.

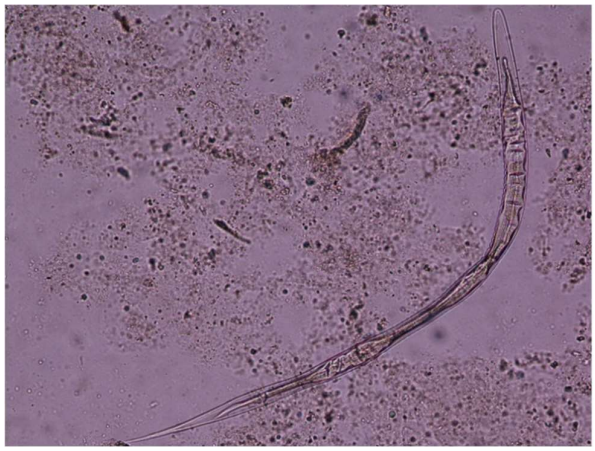

Fig. 5: L_3 was damaged by *A. annua*, aqueous extracts prepared by the decoction method.

Table 3: *In vitro* nematocidal effect of aqueous extracts of *Artemisia* species against *H. contortus* larvae.

	Mean Rank					
Plant extract	1 hr	2 hr	3 hr	6 hr	8 hr	12 hr
ANN-Dec	17.83	18.58	18.92	17.08	19.33	20.42
ABST-Dec	13.50	14.42	14.08	15.00	13.67	14.08
AINC-Dec	15.67	14.67	15.08	14.67	14.42	14.00
ABR-Dec	17.50	17.67	16.67	17.33	17.33	16.17
ATOR-Dec	13.00	12.17	12.75	13.42	12.75	12.83
ANN-M	18.33	20.00	17.50	16.08	18.42	15.50
ABST-M	15.83	15.83	16.75	17.58	15.33	16.67
AINC-M	16.67	13.83	13.92	13.17	14.00	14.50
ABR-M	13.17	16.08	16.17	16.92	16.00	17.00
ATOR-M	13.50	11.75	13.17	13.75	13.75	13.83
Pbs	0	0	0	0	0	0
	Test Statistics[a,b]					
Asymp. Sig.[c]	.822	.716	.778	.928	.669	.588
Asymp. Sig [d]	.832	.573	.897	.882	.896	.966

a. Kruskal Wallis Test, b. Grouping Variable: Plant, c. Decoction extract, d: Maceration extract: ABST: *A. absinthium* L., ABR: *A. abrotanum* L., ANN: *A. annua* L., AINC: *A. incana* (L.) Druce, ATOR: *A. tournefortiana* Rchb: Dec: aqueous extract prepared by decoction method, M: aqueous extract prepared by maceration method

aqueous extracts showed an anthelmintic activity on *H. contortus* larvae comparable to the traditional anthelmintic agent albendazole, increasing with concentration (1.5625-50 mg/mL). Among the studied species, it was observed that *A.annua* was the most effective and *A. tournefortiana* was the least effective species against larvae. *In vivo* and *in vitro* studies demonstrated anthelmintic activities of the extracts obtained from different *Artemisia* species against *H. contortus* (Iqbal *et al*., 2004; Zhu *et al*., 2013; Irum *et*

al., 2015; Karim *et al*., 2019; Ahmed *et al.,* 2020; Higuera-Piedrahita *et al*., 2021; Higuera-Piedrahita *et al*., 2022).

In a study describing the *in vitro* and *in vivo* anthelmintic activity of *Artemisia brevifolia*, crude aqueous (CAE) and methanol extracts (CME) of the whole plant had an anthelmintic effect on live *H. contortus*. This effect was demonstrated by their paralysis and/or mortality at 6 hours after exposure, according to in vitro experiments (Iqbal *et al*., 2004). The paralysis and/or death of live adult *H. contortus* worms were observed as significant anthelmintic effects of crude aqueous and ethanolic extracts of the aerial portions of *A. absinthium* at various time intervals after treatment (Tariq *et al*., 2008). *A. lancea*'s essential oil (EO) exhibited some anthelmintic effect against the eggs and larvae of *H. contortus* (Zhu *et al*., 2013). Following the post-treatment period, the fecal egg count (FEC) significantly decreased for *Artemisia vestita* and *Artemisia maritima*. On day 28 post-treatment, the maximum reduction in fecal egg count for *A. vestita* was 87.2% at 100 mg/kg, whereas for *A. maritima* it was 84.5%. Examined extracts showed a high level of efficacy against larvae and adults (Irum *et al*., 2015). As determined by the reduction of egg hatching and the mobility of larvae and adult worms, *Artemisia vulgaris*' crude aqueous and ethanolic extracts exhibited considerable ($p<0.05$) in vitro anthelmintic efficacy against *H. contortus*. Compared to the aqueous extract, the ethanolic extract exhibited more anthelmintic action (Karim *et al*., 2019). The larvae and adult stages of *H. contortus* exhibited decreased hatching and reduced motility in response to the in vitro anthelmintic activity of *Artemisia herba-alba* (Ahmed *et al*., 2020). In a study examining the anthelmintic activities of *A. absinthium* and *Malva sylvestris* L. in 2020, similar to our study, it was reported that aqueous extracts of the plants showed a strong nematocidal effect (ED50 values of 1.40 and 3.76 mg/mL, respectively) on *H. contortus* eggs (Mravčáková *et al*., 2020). In another study conducted with ethanol and aqueous extracts of *A. campestris* L., it was observed that the anthelmintic activity of both extracts increased in a dose-dependent manner on eggs and adults of *H. contortus*. Both extracts completely inhibited egg hatching at a concentration of about 2 mg/mL. The LD50 values of *A. campestris* ethanolic and aqueous extracts were reported as 0.83 and 1.00 mg/mL, respectively ($p < 0.05$). At the highest concentration tested, after 8 and 24 hours of exposure to the extracts, it was reported that the ethanol extract had a nematocidal effect of 91.3% and 100%, and the aqueous extract of 3.22% and 70.96%, respectively (Akkari *et al*., 2014). Our findings are compatible with this literature.

In a study conducted in 2022, ethyl acetate (EAE) and aqueous (AQE) extracts of *A. absinthium* were prepared, total contents of phenolic compounds, flavonoids and condensed tannin were measured (Hbika *et al*., 2022). EAE showed a high polyphenol and flavonoid content, while AQE had a higher concentrated tannin content. As a result of the preparation of *Artemisia* species with different solvents, the chemical substances responsible for the activity may vary. It was thought that positive results could be obtained as a result of examining the anthelmintic activities of the extracts of different *Artemisia* species to be prepared with different solvents.

In a study, the anthelmintic effects of *Artemisia cina* extracts prepared with n-hexane, ethyl acetate and methanol on *H. contortus* eggs were investigated and it was reported that the highest activity was seen with the hexane extract (Higuera-Piedrahita *et al*., 2021). Later, the hexane extract of *A.cina* was also found to be effective on *Teladorsagia circumcincta* eggs (Higuera-Piedrahita *et al*., 2022). In our study, only five *Artemisia* species (*A. absinthium, A. abrotanum, A. annua, A. incana* and *A. tournefortiana*) were studied. Anthelmintic activity was also detected with different *Artemisia* species, e.g., *A.abyssinica, A. afra, A. herba-alba, A. santonica,* and *A. sogdiana* (Higuera-Piedrahita *et al*., 2022). Considering these results, it can be thought that the studied species may have similar chemical structures responsible for the anthelmintic effect.

Conclusions: As a result, it can be said that all of the studied extracts showed a higher anthelmintic effect against *H. contortus* eggs than their effects on their larvae. As far as we know, this is the first study to scientifically evaluate the anthelmintic activities of *Artemisia* species that grow naturally in Türkiye and are used as anthelmintic by the local people, but *in vitro* studies should be developed with *in vivo* studies. This study showed that ethnobotanical data can be a starting point for clinical studies. In particular, the high inhibition effect observed on eggs should be investigated in more detail. By identifying the active ingredients responsible for the activity, it is possible to bring anthelmintic plant extracts and active ingredients to the literature.

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