

Research Article

Ovocidal Effect of *Achillea salicifolia* and *Hedysarum Gmelinii* Extracts on *Toxocara Cati* Eggs in an *in Vitro* Experiment

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Abstract: This study investigated the ovicidal activity of *Achillea salicifolia* and *Hedysarum gmelinii* extracts against *Toxocara cati* eggs under *in vitro* conditions. Two concentrations (5% and 10%) and four exposure times (0.5, 1, 24, 48 h) were tested in triplicate. The proportion of viable and non-viable eggs, embryo destruction, and shell degeneration were recorded microscopically. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$). At early exposure (0.5–1 h), all treatments showed high viability ($\geq 60\%$) with no significant differences from the control ($p > 0.05$). After 24 h, egg non-viability markedly increased in both plant extracts: *A. salicifolia* 5% = $81.0 \pm 3.5\%$, 10% = $88.0 \pm 2.9\%$; *H. gmelinii* 5% = $77.0 \pm 3.8\%$, 10% = $82.0 \pm 3.3\%$. Embryo destruction reached 45–52%, and shell degeneration 40–48%. At 48 h, maximal ovicidal effects were observed: *A. salicifolia* 10% = $89.0 \pm 2.7\%$ non-viable eggs ($p < 0.01$), embryo destruction $60 \pm 4\%$, shell degeneration $55 \pm 5\%$; *H. gmelinii* 10% = $87.0 \pm 3.1\%$ ($p < 0.05$), embryo destruction $57 \pm 4\%$, shell degeneration $51 \pm 4\%$. Phenol (4%) produced the highest ovicidal effect ($94.0 \pm 1.2\%$ non-viable, $p < 0.001$), whereas the untreated control maintained $>90\%$ viability. The results demonstrate a clear time- and concentration-dependent pattern. *A. salicifolia* exhibited faster onset of action, while *H. gmelinii* showed slower, cumulative effects associated with progressive embryonic destruction and shell degradation. Both extracts displayed moderate but significant ovicidal activity, highlighting their potential as environmentally friendly botanical alternatives to conventional chemical ovicides for managing *Toxocara* contamination.

Keywords: *Toxocara cati*, Ovicidal Activity, *Achillea salicifolia*, *Hedysarum gmelinii*, Plant Extracts, Anthelmintic, Botanical Pesticide, *In Vitro*

Introduction

Toxocara cati is one of the most widespread helminths in domestic and stray cats (Overgaauw and van Knapen, 2013). This parasite poses a significant

zoonotic threat, especially to children, who may become infected through contact with contaminated soil, sand or animal hair (Despommier, 2003). *T. cati* eggs excreted with feces are able to persist in the environment (soil, litter, surfaces) for a long time due

to a strong multilayer shell that protects against adverse factors (Kong and Peng, 2020). In such conditions, eggs can remain invasive for up to several years, contributing to the steady circulation of the pathogen in nature and the environment (Pereira *et al.*, 2016).

Recent studies conducted in Kazakhstan indicate that the helminth *T. cati* was the predominant species of gastrointestinal nematode identified in the feline population. The overall prevalence of egg shedding was determined to be 6.2% across five cities in Kazakhstan. Notably, the prevalence of *T. cati* egg shedding varied significantly among the surveyed Kazakhstan cities. For instance, this metric was 15.2% in Almaty and 2.4% in Astana (Lider *et al.*, 2025).

Control of the spread of *T. cati* requires a comprehensive approach, including deworming of animals, sanitary and hygienic measures and disinfection of environmental objects (Bauer *et al.*, 2024). Inactivation of eggs in the environment is particularly difficult, since most chemical ovocides are either not effective enough or have toxic effects on animals, humans and ecosystem components (Ng'etich *et al.*, 2023). One of the most effective agents is considered phenol, but its use is limited by its high toxicity, corrosiveness and pronounced environmental contamination (Gucbilmez, 2022).

In this context, the search for a biologically safe and effective alternative to chemical disinfectants is a pressing (or critical) task. Plant-derived preparations exhibiting anthelmintic properties, including ovocidal activity, are of particular interest (Oliveira *et al.*, 2019; Cortes-Morales *et al.*, 2022). Plants containing flavonoids, saponins, terpenes, glycosides, alkaloids, tannins, essential oils, and other biologically active substances have anthelmintic activity and have the potential to disrupt the permeability of helminth eggshells, inhibit germ metabolism, or induce their death (Adak and Kumar, 2022).

Achillea salicifolia is a perennial plant of the family *Asteraceae*, widespread throughout Kazakhstan. The plant contains alkaloids, flavonoids, vitamin K, carotene, tannins and other biologically active substances. The main chemical components of the essential oil of the plant are terpenoids. Extracts of *A. salicifolia* are active against *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Clostridium difficile* strains, and have cytotoxic, antioxidant properties (Asadullaeva *et al.*, 2024).

Saponins isolated from plant material have molluscocidal, anthelmintic and cytotoxic activities. The crude *A. wilhelmsii* saponins (CSA) had cytotoxic activity with LC₅₀ values of 2.3±0.16 µg/mL respectively. For *in vitro* anthelmintic activity, paralysis time and parasite death (antiparasitic activity) were observed. At 40 mg/mL concentration, crude saponins of *A. wilhelmsii* were 1.96

and 2.12 times more potent than albendazole against *Pheretima posthuma* and *Raillietina spiralis*, respectively (Ali *et al.*, 2011).

In vitro studies revealed the anthelmintic effect of aqueous and ethanol extracts of *A. millefolium* L. on live worms *Haemonchus contortus* (p<0.05), as evidenced by their paralysis and death in 8 h after exposure. While aqueous extracts of *A. millefolium* resulted in an average inhibition of worm motility of 94.44%, ethanolic extracts caused an average inhibition of worm motility of 88.88% (Tariq *et al.*, 2008).

Extracts of *A. fragrantissima* flowers, rich in phenols, flavonoids and tannins, caused significant inhibition (100%) of the sporulation process for *E. papillata* oocysts when exposed to 300 and 200 mg/mL (Al-Shaebi *et al.*, 2023).

The genus *Hedysarum* contains flavonoids, saponins, and other biologically active compounds which the literature links to anthelmintic activity in plants. This substantiates the hypothesis concerning a possible ovocidal effect (Dong *et al.*, 2013). Certain analogous species within the genus *Hedysarum* are cited as exhibiting anthelmintic properties (Amato *et al.*, 2016).

Hedysarum gmelinii is a promising plant species for introduction into culture and further use in agro-industrial complex and pharmacology. Biologically active compounds of the plant are localized mainly in the root system. From *H. gmelinii* flavonoids (2.6%) 3-α-L-rhamnofuranosidequercetin, 3-α-L-arabinofuranosidequercetin; chalcones hedizarumin A, hedizarumin B, paratocarpin E; pterocarpin 3-hydroxy-9-methoxypterocarpin were obtained; triterpenoids lupeol, soyasapogenol, squazapogenol; coumarin 3,9-dihydroxycoumestane; sterol β-sitosterol; palmitic acid, and 2,3-dihydroxypropyl ester of hexadecanoic acid.

In the present study, *H. gmelinii* is considered a promising source of natural bioactive compounds. However, a significant portion of the existing literature is primarily dedicated to phytochemical screening and the study of general pharmacological effects, rather than ovocidal (anthelmintic) activity. Scientific papers have successfully isolated and structurally characterized novel prenylated chalcones and flavonoids, which demonstrated anti-inflammatory, anti-proliferative, and cytotoxic activities in cell models; these results substantiate the species' pharmacological potential (Liu *et al.*, 2018; Dong *et al.*, 2013; Khan *et al.*, 2013).

However, the anthelmintic properties as well as ovocidal activity of *H. gmelinii* are not sufficiently studied.

In this work, we consider extracts of *Achillea salicifolia* and *Hedysarum gmelinii* as promising natural agents for disinfection of environmental

objects contaminated with *Toxocara cati* eggs.

The aim of this study was to evaluate the efficacy of *Achillea salicifolia* and *Hedysarum gmelinii* extracts for inactivation of *Toxocara cati* eggs under *in vitro* conditions.

Materials and Methods

This study was conducted from February 2024 to June 2025 at the Kadyrov Laboratory of Parasitology in the Department of Veterinary Medicine, Faculty of Veterinary and Animal Husbandry Technology, S. Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan

Mature eggs of *T. cati* from infected cats were used for the study. Fecal samples were collected from 245 cats admitted to the “Kovcheg” cat shelter (Astana city, Kazakhstan) to obtain *T. cati* eggs. These cats were spontaneously infected with the pathogen and had not undergone anthelmintic treatment. The *T. cati* eggs are nearly spherical, measuring $65 - 75 \times 75 - 85 \mu\text{m}$, and possess a thick, brown shell with a granular, rough, bumpy surface and a dark, uniformly granular content (Jenkins, 2020). Microscopic evaluation was then performed in Figure (1) using the direct Sheather’s sugar flotation method with a sugar solution of specific gravity 1.3 to detect helminth eggs (Kassai, 2013). After flotation, *T. cati* eggs were washed thoroughly with physiological solutions and the eggs were collected in sterile tubes. The obtained biomaterial was centrifuged and washed again with distilled water to remove organic impurities and necrotic tissues.

The extracts of *A. salicifolia* and *H. gmelinii*, prepared by extraction method using Soxhlet KEX 250 extractor (Germany) from dried and crushed plant material (herb, roots) were used as the studied agents; ethanol with chloroform was used as extractants.

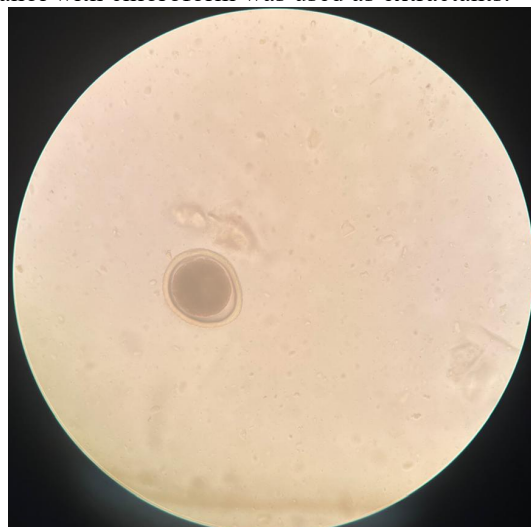


Fig. 1: Eggs of *Toxocara cati* from the family *Ascarididae*

Subsequent distillation of the primary extract on a rotary evaporator (IKA HB digital, Germany) made it possible to obtain thick extracts.

The extraction of the plant extract was performed using standard specialized equipment – an extractor. The plant material to solvent ratio was 1:10. Ethanol and chloroform were used as solvents in a 1:1 ratio. The extraction time was 3h (The State Pharmacopoeia of the RK, 2008).

The extractor vessel, designed for substance recovery, was heated to the boiling point of water. The steam, upon entering the central tube of the reflux condenser, condensed there, and the resulting liquid (extract) flowed into the wide section of the extractor. When the level of the liquid containing the dissolved active substances (the extract) in the extractor reached the bend of the siphon tube, the extract drained back into the boiling flask via the tube. This all ows for the continuous dissolution of the compounds, which, along with the solvent, returns to the flask where the extract becomes increasingly concentrated while the overall volume of the liquid remains practically constant. This mechanism enables the recovery of a large quantity of the extractable substance using a limited volume of solvent.

For the extraction, the raw plant material was placed inside the extractor, encased in a clean glass fiber material. The extractor was charged with water until the liquid began to cycle, flowing through the siphon tube into the boiling vessel. The reflux condenser was then mounted onto the extractor, with the caps of both units being tightly fastened with screws. Cooling water circulation was initiated through the condenser. Finally, the integrity of all connections and fastenings was confirmed before starting the heating phase.

A safety mechanism is incorporated into the system to automatically deactivate the heating element and thus prevent acid volatilization into the environment, should the flow of cooling water to the reflux condenser cease.

The principal aim of this investigation was to assess the total biological activity exhibited by the resulting extract. Therefore, we conducted quality control of the extracts based on their organoleptic, hygienic, chemical, and microbiological parameters, in accordance with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan (ST RK 978-2001, 2001).

From the thick plant extracts, by dilution in dimethyl sulfoxide solution, liquid forms of *A. salicifolia* and *H. gmelinii* extracts at concentrations of 5 and 10% were further prepared and tested to determine their inactivation capacity against *T. cati* eggs. The 5 and 10% solutions were prepared as mass percentages (w/v) by dissolving 5 g and 10 g of the viscous extract in 100 mL of dimethyl

sulfoxide, respectively.

A 4% phenol solution was used as a chemical control. *Toxocara* eggs incubated exclusively in physiological saline served as the negative control. Eggs of each variant (experimental and control groups) were placed in individual wells of flat polystyrene plates 0.5 ml each, adding the appropriate solutions. Incubation was carried out at 25°C for four different time intervals: 30 min, 60 min, 24 h and 48 h. At the end of the exposure, the eggs were washed three times with distilled water to remove residual solution and then proceed to microscopic evaluation. The experiments were conducted in triplicate, utilizing 100 ± 3 *Toxocara cati* eggs per replicate.

The assessment of viability relied upon morphological features detailed in prior research publications. (Abou-El-Naga, 2018). Egg viability was assessed using light microscopy ($\times 400$ magnification) by observing embryo activity (movement within the shell) and morphological integrity. Eggs showing signs of embryo destruction, lack of movement and loss of clarity of contours were classified as non-viable. At least 100 eggs per replicate were counted for each time interval and each solution tested. The results were expressed as the percentage of viable and non-viable eggs from the total number of eggs examined.

Statistical analysis of the data was performed using the Statistica 13.0 and SPSS software packages. Prior to selecting the appropriate statistical test, data distribution was assessed using the Shapiro–Wilk test. For data

showing a normal distribution, one-way analysis of variance (ANOVA) was applied, followed by Tukey's post-hoc test for pairwise comparisons.

For data that did not follow a normal distribution, the Kruskal-Wallis's test was used, followed by Dunn's post-hoc test.

Results were considered statistically significant at $p < 0.05$. The levels of significance were indicated as follows: ns not significant ($p > 0.05$); $p < 0.05$; $p < 0.01$; $p < 0.001$.

Results

The study involved a comparative evaluation of the ovicidal activity of *Achillea salicifolia* and *Hedysarum gmelinii* extracts against *Toxocara cati* eggs under controlled *in vitro* conditions. A 4% phenol solution was used as a positive control, while an untreated batch of eggs served as the negative control.

Summary data on egg viability and microscopic evaluation results, including parameters such as embryo destruction and shell degeneration, are shown in table 1.

At the early stages of exposure (0.5–1h), egg viability (% Viable) remained relatively high across all treatment groups. The differences between plant extracts and the negative control were statistically insignificant (*ns*, $p > 0.05$) (Table 1).

This outcome corresponds to the short exposure time and the low permeability of the eggshell to active components. Microscopically, only minor signs of embryonic damage and slight shell softening were detected (Table 1), confirming the limited ovicidal potential of the tested extracts at the initial stages of exposure.

Table 1: Evaluation of the ovicidal activity of *Achillea salicifolia* and *Hedysarum gmelinii* extracts against *Toxocara cati* *in vitro* (n=3)

Treatment	Concentration	Time (h)	Nonviable eggs \pm SD (%)	Embryo destruction \pm SD (%)	Shell degeneration \pm SD (%)	ANOVA p (Nonviable, same time)	Significance vs Control (Nonviable)
<i>Achillea salicifolia</i>	10%	0.5	43.0 \pm 5.0	20.3 \pm 4.7	22.7 \pm 6.1	0.0341	—
<i>Achillea salicifolia</i>	5%	0.5	37.0 \pm 5.0	17.7 \pm 2.5	19.3 \pm 4.6	0.0341	—
<i>Hedysarum gmelinii</i>	10%	0.5	28.0 \pm 5.0	18.0 \pm 3.6	10.0 \pm 1.7	0.0341	—
<i>Hedysarum gmelinii</i>	5%	0.5	31.0 \pm 6.0	15.0 \pm 4.0	16.0 \pm 5.3	0.0341	—
<i>Achillea salicifolia</i>	10%	1.0	58.7 \pm 5.5	22.0 \pm 4.4	36.7 \pm 7.6	0.0617	—
<i>Achillea salicifolia</i>	5%	1.0	50.0 \pm 5.0	19.7 \pm 5.0	27.0 \pm 6.1	0.0617	—
<i>Hedysarum gmelinii</i>	10%	1.0	47.0 \pm 6.0	24.7 \pm 8.1	22.3 \pm 3.5	0.0617	—
<i>Hedysarum gmelinii</i>	5%	1.0	44.7 \pm 5.5	21.3 \pm 7.8	23.3 \pm 5.8	0.0617	—
<i>Achillea salicifolia</i>	10%	24.0	88.0 \pm 3.0	23.3 \pm 2.1	64.7 \pm 5.0	0.0240	—
<i>Achillea salicifolia</i>	5%	24.0	81.0 \pm 4.0	40.3 \pm 7.2	40.7 \pm 8.0	0.0240	—
<i>Hedysarum gmelinii</i>	10%	24.0	79.0 \pm 3.0	37.0 \pm 16.1	42.0 \pm 18.4	0.0240	—
<i>Hedysarum gmelinii</i>	5%	24.0	77.0 \pm 4.0	42.7 \pm 13.1	34.3 \pm 15.7	0.0240	—
<i>Achillea salicifolia</i>	10%	48.0	90.0 \pm 5.3	47.3 \pm 15.5	42.7 \pm 11.0	0.0000	***
<i>Achillea salicifolia</i>	5%	48.0	89.0 \pm 3.0	32.0 \pm 9.0	60.3 \pm 7.8	0.0000	***
<i>Hedysarum gmelinii</i>	10%	48.0	87.0 \pm 3.0	42.3 \pm 11.0	44.7 \pm 8.5	0.0000	***
<i>Hedysarum gmelinii</i>	5%	48.0	85.0 \pm 3.0	46.3 \pm 19.5	38.7 \pm 20.1	0.0000	***
Phenol (positive control)	4%	48.0	94.0 \pm 1.0	48.0 \pm 22.5	46.0 \pm 22.6	0.0000	***

Note: Values are presented as mean \pm standard deviation (SD) from three replicates for each “group \times time” combination. Statistical testing was performed for % Nonviable eggs at each time point using one-way ANOVA; when a control group was included, Tukey's post-hoc comparisons were conducted only against the negative control. Statistical significance is indicated by asterisks as follows: $p < 0.05$; $p < 0.01$; $p < 0.001$; ns not significant.

By 24 hours, the percentages of nonviable eggs, embryo destruction, and shell degeneration increased significantly in all extract-treated groups, particularly at 10 % concentrations of both *A. salicifolia* and *H. gmelinii*. However, the intergroup variation remained moderate, and the statistical significance ranged from *ns* to $p < 0.05$, depending on concentration and parameter. These results reflect the gradual manifestation of toxic effects linked to the cumulative action of bioactive constituents over time.

At 48 hours, the effects reached their maximum expression (Table 1). The 10 % *A. salicifolia* extract produced a marked increase in the proportion of nonviable eggs and embryo destruction ($p < 0.05$ $p < 0.01$), demonstrating a clear dose- and time-dependent pattern. A similar tendency was noted for *H. gmelinii*, although the degree of shell degradation was slightly less pronounced. The positive control (4 % phenol) induced almost complete embryonic mortality and severe structural disintegration of the shell, confirming both the accuracy of the experimental model and the high sensitivity of the test system. The negative control maintained more than 90 % viability, with intact shells and minimal embryonic alterations.

The morphological appearance of *T. cati* eggs after treatment with 10 % extracts of *A. salicifolia* and *H. gmelinii* is illustrated in Figs 2 and 3, respectively. Microscopic observation revealed distinct destructive changes: partial or complete rupture of the outer shell, disintegration of the internal contents, confirming the progressive destruction of embryonic material.

Overall, the statistical analysis (one-way ANOVA followed by Tukey's post-hoc test) confirmed significant differences between the treated and control groups at later time points (24 – 48h), especially for phenol and the 10 % plant extract concentrations. Both extracts exhibited a pronounced but moderate ovicidal activity dependent on concentration and exposure duration. These findings indicate that prolonged exposure enhances the effectiveness of the plant-based preparations, validating their potential as environmentally safe alternatives to synthetic ovicidal agents.

Discussion

The main objective of this study was to assess the ovicidal effect of *Achillea salicifolia* and *Hedysarum gmelinii* extracts on *Toxocara cati* eggs.

As reported in the literature, the pharmacological properties of *H. gmelinii* preparations including its bactericidal, antioxidant, anti-inflammatory, and immunostimulatory activities are attributed to its biologically active components.

These constituents encompass flavonoids (e.g., hyperoside), anthocyanins, and resins (Yurkevich *et al.*, 2024; Liu *et al.*, 2018; Dong *et al.*, 2013). Phytochemical analyses of the genus *Hedysarum* in literature reviews confirm the presence of over 155 biologically active

compounds, including flavonoids, triterpenes, and coumarins (Farajpour *et al.*, 2013). The inherent general antiparasitic potential attributed to this genus suggests that *H. gmelinii* may possess ovicidal activity. Nevertheless, targeted *in vitro* studies are critically required to verify the specific ovicidal activity of *H. gmelinii* extracts and to isolate the key active metabolites necessary for the development of phytopharmaceuticals (Szewczuk *et al.*, 2003; Farajpour *et al.*, 2013).

The study by Saraç *et al.* (2021) aimed to determine the concentrations of macro- and microelements as well as the antioxidant activity of an aqueous extract of *A. millefolium* L. (yarrow), which is an important medicinal plant. The results demonstrated that yarrow contains sufficient levels of certain macroelements (N, P, K, Ca, Mg) and microelements (Fe, Zn, Mn, Cu) and exhibits moderate antioxidant potential. Furthermore, the plant's Oxidative Stress Index (OSI) was found to be low (Saraç *et al.*, 2021).

The mineral profile of 25 diverse accessions across three *Achillea* (yarrow) species was investigated to uncover the genetic factors influencing elemental accumulation. Analysis revealed significant intra- and interspecific variability in macro- and microelement content.

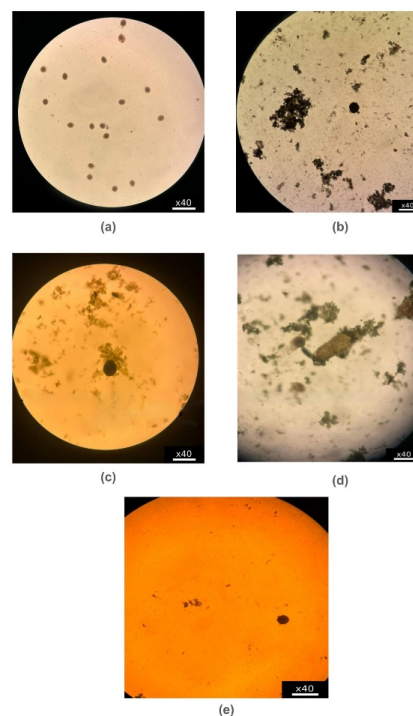


Fig. 2: Signs of destruction egg of *Toxocara cati* after exposure to *Achillea salicifolia* 10% as a function of exposure time (x40); a: *Toxocara cati* eggs before treatment with extract, microscopy x40, b: Dead eggs of *Toxocara cati* after 30 min exposure to *A. salicifolia* 10%, c: Dead eggs of *Toxocara cati* after 60 min exposure to *A. salicifolia* 10%, d: Dead eggs of *Toxocara cati* after exposure to *A. salicifolia* 10% for 24 h, e: Dead eggs of *Toxocara cati* after exposure to *A. salicifolia* 10% for 48 h

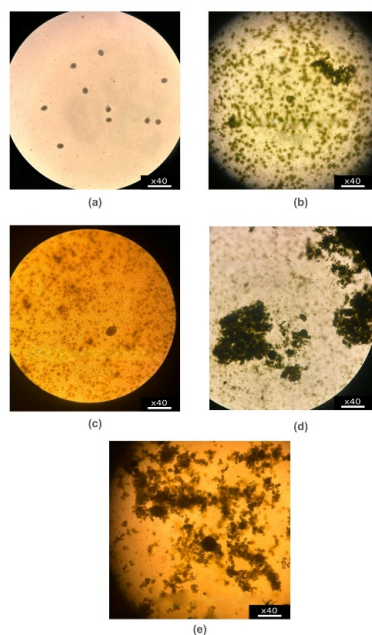


Fig. 3: Signs of destruction egg of *Toxocara cati* after exposure to *Hedysarum gmelinii* 10% as a function of exposure time (x40); a: *Toxocara cati* eggs before treatment with extract, microscopy x40, b: Dead eggs of *Toxocara cati* after 30 min exposure to *H. gmelinii* 10%, c: Dead eggs of *Toxocara cati* after 60 min exposure to *H. gmelinii* 10%, d: Dead eggs of *Toxocara cati* after exposure to *H. gmelinii* 10% for 24 h, e: Dead eggs of *Toxocara cati* after exposure to *H. gmelinii* 10% for 48 h

This compellingly indicates that genotype plays a pivotal role in determining the nutritional and therapeutic value of these wild medicinal plants. (Farajpour *et al.*, 2024). Extracts and essential oils derived from *A. millefolium* demonstrated activity against several microbial species, including the bacteria *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*, as well as the fungus *Candida albicans* (Falconieri *et al.*, 2011).

Our findings establish that the tested botanical extracts exhibit significant ovocidal efficacy against *T. cati* eggs, thus validating their promise as a sustainable alternative to synthetic chemical control agents. Specifically, the extract of *A. salicifolia* displayed the most potent effect, achieving levels of ovocidal action that were nearly equivalent to the reference compound, phenol. Crucially, the substantial reduction in egg viability observed even at lower extract concentrations suggests a strong presence of highly biologically active metabolites. The kinetics of egg inactivation for the *H. gmelinii* extract exhibited a distinct pattern: differences across varying concentrations were minimal, whereas exposure time emerged as the critical factor. This observation suggests that the ovocidal effect of *H. gmelinii* compounds is achieved through a time-dependent mechanism, requiring prolonged contact with

the parasite eggs to gradually compromise protective structures and inhibit embryonic development, as supported by the data shown in Table 1, where the proportion of nonviable eggs increased sharply between 24 and 48 hours.

A crucial observation is the dependence of the ovocidal effect on exposure duration: activity was minimal during short contact times of 0.5 – 1 h but dramatically increased by 24 – 48 h. This finding aligns with literature suggesting that plant metabolites disrupt the eggshell and embryonic tissues only upon prolonged contact, leading to osmotic imbalance and structural degradation (Sundararajan *et al.*, 2018; Delgado-Núñez *et al.*, 2023).

Analysis of the possible mechanisms of action suggests differences between the investigated plant species. For *A. salicifolia*, key roles may be attributed to its flavonoids, tannins, and essential oils, compounds known for their ability to penetrate lipid membranes and increase the permeability of the helminth's protective envelopes (Garcia-Bustos *et al.*, 2019). In contrast, the leading compounds in *H. gmelinii* are likely triterpenoids, saponins, coumarins, and flavonoids, which are hypothesized to disrupt the integrity of the protective barriers and consequently inhibit embryonic development (Dong *et al.*, 2013).

The flavonoid content in the extracts of *H. gmelinii* and *A. salicifolia* is implicated in affecting diverse intracellular signaling cascades of the oocyst. Functionally, these compounds can regulate and inhibit the parasites' growth and viability, potentially by triggering apoptosis (Liu *et al.*, 2018; Saadat *et al.*, 2024). Furthermore, the *H. gmelinii* extract is known to contain anthocyanins, which may exhibit cytotoxicity at high concentrations (5% and above), potentially damaging the cells within the parasite eggs. Conversely, at low concentrations, they might show no effect or even exert a cytoprotective action. Salicylic acid, also found in *H. gmelinii*, possesses cytotoxic effects at high doses, consistent with its nature as a toxic compound. Additionally, acetic acid, present in the medicinal formulation of this plant, can diffuse across the parasite egg's cell wall, leading to its acidification and functional impairment. Moreover, acetic acid may act as a trigger for oocyst apoptosis (Egorova *et al.*, 2015).

Thus, the comparative assessment demonstrated that while phenol remains the fastest and most potent ovocidal agent (Zhang *et al.*, 2020), the plant extracts of *Achillea salicifolia* and *Hedysarum gmelinii* exhibit comparable activity upon prolonged exposure and possess an undeniable advantage in terms of environmental safety.

Conclusion

The conducted study demonstrated high ovocidal

activity of both the chemical agent (phenol) and the plant extracts of *Achillea salicifolia* and *Hedysarum gmelinii* against the eggs of the nematode *Toxocara cati*. The plant-derived preparations showed a comparable effect with increased concentration and exposure time.

The observed characteristics of the action indicate a difference in Mechanisms the *A. salicifolia* extracts demonstrated a rapid and pronounced effect as early as 24h, whereas *H. gmelinii* only achieved its maximum activity toward the end of the 48h, period. This kinetic distinction underscores the significance of selecting not only the concentration but also the duration of treatment to achieve optimal results.

The obtained data expand the understanding of medicinal plants' potential as sources for disinfective/decontaminating agents. Owing to their accessibility and relative environmental safety, the extracts of *A. salicifolia* and *H. gmelinii* can be considered a promising alternative to chemical ovocidal drugs. Their application is particularly relevant in settings where there is a necessity to reduce the toxic load on the environment and ensure safety for both animals and humans.

The practical significance of these results lies in the potential for utilizing these plants in the development of novel phytopharmaceuticals for the decontamination/disinfection of external environmental surfaces. Furthermore, it supports their integration into prophylactic measures against *Toxocara cati* in both veterinary and sanitary practice.

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Author's Contributions

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Ethics

This is the original work and contains unpublished material. All the authors have read and agreed to the published version of the manuscript.

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