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Preliminary screening of plant essential oils from an oceanic climate zone (NW Spain) for the control of equine cyathostomins

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Abstract
Cyathostomins (Strongylida: Cyathostominae) are gastrointestinal nematodes (GIN) that affect equines in many parts of the world. Due to anthelmintic resistance (AR), it is necessary to search for control alternatives. The objective of the study was to carry out a screening of 26 essential oils (EOs) from plants to determine in vitro their action on cyathostomins. Essential oils were obtained by hydro-distillation from leaves, flowers, fruits, and seeds of the selected species and used against the eggs of cyathostomins by means of the tests of egg hatching and larval motility inhibition. For each EO, different concentrations were prepared (250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, 2.0, 1.0, 0.5 and 0.24 mg/mL). Oxfendazole at 2.5% mixed with closantel 5% was used as positive control, and PBS served as negative control. Cyathostomin eggs were obtained from feces of infected horses and infective larvae were recovered after coproculture. The in vitro test was carried out 36 h after the challenge with the EOs. The highest production of EOs was obtained from leaves of bay, eucalyptus, mallow, and basil, as well as from seeds of black pepper, coriander, cinnamon, anise, and peel of orange and cloves, cumin, and Lawson's cypress. The oils extracted from laurel leaves, ground cinnamon, anise, cumin, and coriander seeds had a very high effect on cyathostomins eggs up to concentrations lower than 3.9 mg/mL. Therefore, in addition to the uses against other pathogens such as fungi and bacteria, some EOs might attain great importance as an alternative control strategy in the control of nematode eggs.

Article Highlights

- Complementary measures to anthelmintics needed to control cyathostomins affecting horses.
- Nematocidal essential oils can be isolated from plants by hydro-distillation.
- Essential oils obtained from Laurel are antagonists of cyathostomin eggs and larvae.

Keywords Essential oils · Egg hatch test · Motility larval · Cyathostomins · Nematodes

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1 Introduction

Strongyles are the most commonly gastrointestinal nematodes (GIN) affecting equines worldwide [4]. Most known genera include the small strongyles or cyathostomins (*Trichonema*, *Cyalocephalus*, *Cyathostomum* and *Gyalocephalus*) and large strongyles (*Strongylus* and *Triodontophorus*), which are responsible for mild to severe clinical injury [16, 17]. Control of these nematodes involves commercial anthelmintic drugs which are administered to reduce the impact of parasites on animal health and improve their productivity [2]. However, the overuse of different drugs has been pointed as a cause of anthelmintic resistance (AR) identified in the main GIN species, causing low effectiveness [27], thus sustainable control alternatives have been required [31]. For this purpose, several strategies have been counselled, consisting of alternating grazing between horses and sheep [13], targeted and strategic use of anthelmintics [23], and biological control based on nematophagous fungi [18, 26].

More recently, the use of plants with nematicidal action has been evaluated in order to avoid the development of AR. Accordingly, the methanolic extracts or aqueous extracts of leaves and branches taken from different plant species is advised [8, 15]. The use of essential oils (EOs) offers another alternative for the control of GIN, and it has been especially tried in sheep [37]. Several studies investigated the activity of EOs against ovine GIN by means of egg hatch tests [19, 36] or through the larval development test [10]. In addition, some in vivo studies have been carried out on sheep [37]. In horses, carvacrol has been studied as the main component derived from EOs of plants for the control of *Parascaris* [39].

By considering that the EOs of certain plants might contain bio-active molecules that cause the mortality of eggs and larvae of cyathostomins, this appears a sustainable alternative for the control of parasitic nematodes. The objective of the current study was to carry out a screening to determine the possibility of plants located in Galicia (NW Spain) could develop parasiticide activity through their essential oils collected of leaves, seeds, stems, and flowers by means of steam entrainment on eggs and larvae of equine cyathostomins.

2 Materials and methods

2.1 Study localization

The current investigation was developed in Galicia (NW Spain), and the extraction of oils in the city of Lugo, Spain with Coordinates: 43°0′35.7″ North 7°33.361′West. It is a region situated in a mild area with oceanic climate characterized by medium humidity on the coast, higher in the central mountains and drier in the interior. The climate corresponds to temperate with more rain in winter than in summer, with temperature is on average 11.4 °C and about 999 mm precipitation [21]. Accordingly, plant species between Atlantic and Mediterranean vegetation are described [5].

2.2 Selection of vegetal material and essential oils yielding

The screening of products obtained by steam drag and EOs from different plants against eggs and larvae of cyathostomins was carried out especially considering native and introduced species present in Galicia. Several of those reported in the literature with some proven action on GIN, as well as others with EOs although without history of action on the parasite nematodes were also considered. The composition of the oils was not established because the general objective consisted of screening the effectiveness of the steam entrainment products and EOs, on the eggs and larvae of GIN, then selecting those products with high effectiveness, and finally looking for an active ingredient.

Leaves, flowers, fruits of the selected species were collected in the region and some seed were purchased (Table 1). The locally collected species were identified by university herbarium staff. The freshly harvested species were dried at 45 °C for 24 h, then milled in a stainless-steel electric cereal grind (Vevor 350G) to obtain particles < 1 mm thick and finally stored until used for extraction.

The EOs were obtained by hydro-distillation with Clevenger equipment. Between 25 and 60 g of grinded material and 500 mL of distilled water were deposited in a flask. A minimum time of 30 min was left in the extraction process and the volume produced from each plant was recorded. Regardless of the EOs yielding, the supernatant was collected and used in in vitro tests. The weight of the material and also the EOs yielding were recorded for each plant.

Table 1 Production of essential oils of the different plants collected in the region of Galicia, Spain

Date	Plant	Part	Scientific name		Yield	
				G*	mL	By 100 g
23-1-23	Laurel	Leaf	*Laurus nobilis*	73	0.5	0.685
23-1-23	Nettle	Leaf	*Urtica dioica*	38	–	–
20-1-23	Gorse	Leaf	*Ulex europeaus*	25	–	–
20-1-23	Eucalyptus	Leaf	*Eucalyptus globulus*	50	0.25	0.500
23-1-23	Broom	Leaf	*Cytsus scoparius*	60	–	–
25-1-23	Pine	Leaf	*Pinus*	65	–	–
25-1-23	Prickly broom	Leaf	*Genista tridentata*	50	–	–
16-2-23	Black pepper	Seed	*Piper nigrum*	60	2.0	3.333
16-2-23	Holly	Leaf	*Ilex aquifolium*	90	–	–
16-2-23	Mallow	Leaf	*Pelargonium graveolens*	40	0.2	0.500
16-2-23	Olive	Leaf	*Olea europaea*	43	–	–
16-2-23	Silver wattle	Flower	*Acacia dealbata*	50	–	–
20-2-23	Orange peel	Fruit	*Citrus sinensis*	84	0.2	0.238
20-2-23	Cherry laurel	Leaf	*Prunus lauracerasus*	60	–	–
20-2-23	Silver wattle a	Leaf	*Acacia dealbata*	50	–	–
20-2-23	Olive	Fruit	*Olea europaea*	160	–	–
20-2-23	Yew	Leaf	*Taxus baccata*	80	–	–
8-3-23	Clove	Seed	*Syzygium aromaticum*	52	2.5	4.808
8-3-23	Camelia	Leaf	*Camelia japonica*	60	–	–
8-3-23	Basil	Leaf	*Ocimum basilicum*	50	0.1	0.200
13-3-23	Coriander	Seed	*Coriandrum sativum*	50	0.13	0.260
13-3-23	Cinnamon	Stem	*Cinnamomum verum*	50	0.25	0.500
14-3-23	Star anise	Seed	*Illicium verum*	50	0.25	0.500
14-3-23	Cumin	Seed	*Cuminum cyminum*	50	0.6	1.200
14-3-23	Lawson's cypress	Cone	*Chamaecyparis lawsoniana*	61	0.3	0.492

*G: Amount placed in one-liter flask

2.3 Collection and cleaning of cyathostomin eggs

Ten Galician Pure Bred autochthonous horses belonging to the Gayoso farm located in Castro de Ribera de Lea (Lugo, NW Spain) were used. Coprological analysis over more than a decade did show the presence of Strongyles eggs, which were identified as cyathostomins by coproculture [18]. In addition to this, these horses were given ivermectin four months prior to the start of the trial, so only cyathostomin larvae would be expected in the stool cultures, as large strongyles have a longer life cycle [20].

Fecal samples were taken from horses and infection by strongyles demonstrated by the observation of eggs through the McMaster flotation test in saturated saline solution [17, 18]. Those samples with more than 500 eggs per gram of feces (EPG) were taken to concentrate the nematode eggs. This procedure consisted of emulsifying 30 g of feces in 420 mL of tap water and homogenizing properly, then the emulsion was passed through a sieve with a 4-mm diameter corresponding a #100 sieve (0.150 mm, Filtra-vibración, Spain). The filtered liquid with the eggs was recovered in 10 mL glass tubes and centrifuged for ten minutes at 2000 rpm, then the supernatant was discarded, and the sediment dissolved with sucrose solution (d = 1.28), as flotation liquid. The tubes were filled to the brim, on which a glass coverslip was placed, then centrifuged for 10 min at 2000 rpm and the eggs were recovered from the surface of the coverslip. The eggs were washed with distilled water using a Pasteur pipette and placed in 50 mL conical tubes. The egg concentration was estimated from 10 aliquots of 10 µL, adjusting the volume to a final concentration of 2000 eggs/mL.

2.4 Obtaining third-stage larvae of horse cyathostomins

With the aim to get L3 of cyathostomins, a portion of the feces was maintained at 25 °C for 15 days. In concrete, 20 g feces samples were placed into a Petri plate following the Corticelli-Lai stool culture procedure. Subsequently, the larvae

were recovered and concentrated by using the Baerman tube [35]. The larvae were transferred to culture boxes and later used in in vitro tests.

Identification of L3s was performed morphologically by counting the intestinal cells, thus the existence of eight intestinal cells indicated the presence of cyathostomins [25].

2.5 Analysis of the parasiticide activity of EOs

In the present investigation, the parasiticide activity of the EOs obtained was assayed against both eggs and L3 of cyathostomins.

2.5.1 Egg hatching test

The egg hatching assay was developed to determine the products with ovicidal activity. First, a total of 11 Eppendorf tubes were added a volume of 100 µL of water containing 200 cyathostomins eggs each one, then 100 µL EOs at an initial concentration of 1000 mg/mL was added to the first tube, and after proper homogenization, 100 µL of this blend collected (EOs at 500 mg/mL) and transferred to a second tube containing water and 200 eggs, which was mixed, so the EOs were halved (250 mg/mL). In this way, a total of 11 dilutions were made. After that 100 µL of 1% Tween 20 dissolved in PBS were added, so that the final volume was 200 µL and the oils concentrations were 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, 2.0, 1.0, 0.5 and 0.24 mg/mL. In another tube, eggs with Phosphate Buffered Saline + 1% Tween 20 (PBS + T) were placed as the negative control. Positive control samples were prepared by taking 2.5% Oxfendazole mixed with 5% Closantel (Oxydrench, Laboratorios Syva, León, Spain) at an initial concentration of 12.5 and 25 mg/mL, respectively, and then 12 dilutions at half were made. Once the EOs were placed, the tubes were covered and maintained in an incubator at 20 °C for 24 h.

The test was carried out 36 h after the application of the products, the contents of the tube were recovered in five aliquots of 20 µL of each treatment. All wells were checked under an optical microscope at 10X, by counting the total number of unhatched eggs and the number of larvae in first larval stage (L1).

To determine the adjusted mortality, survival in the negative control group (Tween + PBS), the following formula was considered [32]:

$$\text{Adjusted mortality} = 100 - (\text{EOs survival}/\text{Control survival}) \times 100$$

$$\text{Adjusted mortality} = 100 - \left[1 - (\text{Eggs}/\text{Eggs} + L1)_{\text{EOs}} / \, 1 - (\text{Eggs}/\text{Eggs} + L1)_{\text{control}}\right] \times 100$$

where: Eggs = Number of eggs, L1 = number of first larval stage, EOs = Essential oils.

2.5.2 Larval motility test

The cyathostomin larvae recovered from coprocultures were cleaned with distilled water using the Baermann apparatus to separate dead larvae from live ones [38]. The number of larvae was quantified in10 µL aliquots, adjusting the final volume to 100 larvae per 100 µL. In the in vitro tests, the EOs were used in addition to the respective negative control (distilled water), and the positive control was oral Closantel (Endoex, 50 mg/mL, S.P. Veterinaria, Spain). Evaluations were carried out in 96-well polystyrene plates, where approximately 100 larvae contained in 100 µL of water were placed in all plate wells, then 50 µL of EOs (at 1000 mg/mL of initial concentration) + 50 µL PBS with 1% Tween 20 were applied in the first well giving a final concentration of 250 mg EOs/mL. From the first column of the plate 100 µL of each treatment was taken and placed in the second well that already had 100 µL of water with the larvae so the dilution that halved, Subsequently, 100 µL of the mixture were taken from the second well and transferred to the third well, diluting the oil by half, so the final dilutions of the wells were as 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, 2.0, 1.0, 0.5 and 0.24 mg/mL. Closantel

in its commercial presentation was used with an amount of 100 µL at a concentration of 50 mg/µL and serial dilutions were made (25, 12.5, 6.25, 3.1, 1.56, 0.78, 0.39, 0.20, 0.10, 0.05, 0.02, 0.012 mg/µL). Wells with distilled water were used as negative control. Once the EOs were placed, the plate was covered with waxed paper and left in an incubator at 25–26 °C for 24 h. The larval motility test was carried out 24 h after applying the products, taking five aliquots of 20 µL from each well and the number of larvae were counted, sorting between alive and without motility (those that did not show movement for 20 s).

2.6 Statistical analysis

The data obtained from the in vitro study were used to obtain the percentage of effectiveness (larval motility and hatching) for each EO. In addition, the lethal concentration 50 (LC_{50}) and 99 (LC_{99}) were obtained through the Probit analysis with the SAS program [33] based on the following model proposed by SAS user´s guide [33]:

$$\Pr(\text{Response}) = C + (1 - C)\, F(x'\beta) = C + (1 - C)\Phi\left(\beta_0 + \beta_1 \times \log 10(\text{Concentration})\right)$$

where: β: is a vector of estimated parameters. F: is a cumulative distribution function (Normal). X: is a vector of explanatory variables. Pr: is the probability of a response. C: is the natural response rate (proportion of individuals who respond to the zero dose).

3 Results

3.1 Essential oils yield

Laurel leaves, eucalyptus leaves, black pepper seed, mallow leaf, orange peel, cloves, basil leaves, coriander seeds, cinnamon, anise seed, cumin seed, and the cone of Lawson's cypress were the ones that produced essential oils (Table 1).

3.2 Egg hatching test

The EOs extracted from laurel leaves, ground cinnamon bark, anise, cumin, and coriander seeds had a very high effectiveness on cyathostomin eggs up to concentrations lower than 3.9 mg/mL. In the case of Cherry laurel, it was also highly effective despite the fact that EOs was not obtained and only the liquid from the steam drag was used and the dose was considered as the product recovered from the distillation While some plants as broom, olive and holly leaves, as well as mimosa flowers had no effect. The rest of the plants showed some effect on the cyathostomin eggs at different concentrations that are shown in Table 2.

The smallest LC_{50} in the egg hatching corresponded to bay leaves (*L. nobilis)*, mallow (*P. graveolens)*, cherry laurel (*P. lauracerasus*), basil (*O. basilicum*), cumin (*C. cyminum*) and anise seeds (*I. verum*), also cinnamon bark (*C. verum*), and Lawson's cypress (*Chamaecyparis lawsoniana*), while those with high concentrations were from prickly broom (*G. tridentate)* and gorse (*U. europeaus)*, the other plants were in average values. The lethal concentration of the products that had a dose-dependent effect are shown in Table 3.

In general, products with EOs had high mortality in eggs. However, *S. aromaticum* oil had low mortality at the highest concentration used. The oils with the lowest concentration are shown in Fig. 1.

3.3 Larval motility test

The cherry laurel was the only product with more than 90% mortality reached at concentration higher than 63 mg/mL of the supernatant from the steam entrainment of the leaf, although no essential oils were obtained. Also, with a concentration higher than 12.5 mg/mL of Closantel, a larval mortality of 97% was achieved, while the other products provided low mortality percentages at high concentration. Cumin, cinnamon had mortalities higher than 80% with concentrations higher than 7.8 mg/mL and coriander showed high mortality at 250 mg/mL (Table 4).

Table 2 Maximum concentration at which the steam drags products from different plants presented the highest mortality in cyathostomin eggs of horses

Date	Product	Scientific name	Concentration mg/mL	% Mortality*
01/02/2023	Eucalyptus	*Eucalyptus globulus*	250.0	98.8
01/02/2023	Nettle	*Urtica dioica*	250.0	96.8
01/02/2023	Bay	*Laurus nobilis*	3.9	98.5
01/02/2023	Broom	*Cytsus scoparius*	250.0	0.0
01/02/2023	Pine	*Pinus*	250.0	55.2
01/02/2023	Prickly broom	*Genista tridentata*	250.0	15.7
01/02/2023	Gorse	*Ulex europeaus*	250.0	8.4
23/02/2023	Olive leaf	*Olea europaea*	250.0	0.7
23/02/2023	Black pepper	*Piper nigrum*	250.0	93.3
23/02/2023	Silver wattle	*Acacia dealbata*	250.0	4.4
23/02/2023	Mallow	*Pelargonium graveolens*	62.5	93.1
23/02/2023	Holly	*Ilex aquifolium*	125.0	0.7
23/02/2023	Oxydrench		3.0	90.9
06/03/2023	Cherry laurel	*Prunus lauracerasus*	3.9	92.7
06/03/2023	Yew	*Taxus baccata*	7.8	71.2
06/03/2023	Orange peel	*Citrus sinensis*	7.8	88.9
10/03/2023	Basil	*Ocimum basilicum*	31.3	94.0
10/03/2023	Camelia	*Camelia japonica*	31.3	40.6
10/03/2023	Clove	*Syzygium aromaticum*	62.5	92.2
17/03/2023	Star anise	*Illicium verum*	1.0	76.9
17/03/2023	Lawson's cypress	*Chamaecyparis lawsoniana*	2.0	79.8
17/03/2023	Cumin	*Cuminum cyminum*	0.2	88.4
17/03/2023	Cinnamon	*Cinnamomum verum*	1.0	84.0
17/03/2023	Coriander	*Coriandrum sativum*	1.0	77.8

*Mortality adjusted for control with distilled water

The lethal concentration of the products that showed high larval mortality rates at small values are shown in Table 5. Star anise, cumin, and cinnamon were the EOs that showed the best behavior and therefore could be considered for the control of cyathostomin larvae.

4 Discussion

The use of plants in the control of certain pathogens represents an ancient tradition in many cultures, and the usefulness of several species against nematodes and other parasites affecting sheep, goats and horses has recently been reviewed [11, 22, 28]. In the current study, the possibility of phytotherapy to help in the control of cyathostomins infecting horses from Galicia (NW Spain) has been assessed. For this purpose, a total of twenty-five plants were processed by hydro-distillation and essential oils (EOs) collected from twelve of them. The anthelmintic activity was measured against eggs and third-stage larvae of cyathostomins, and then compared to that obtained with two commercial dewormers (Oxfendazole and Closantel). The EOs collected from eucalyptus, laurel, basil, black pepper, mallow, and clove presented a similar or higher ovicidal effect to the chemical anthelmintics (= 90.9% egg-mortality), but none of the EOs attained the larvicidal effect recorded with the commercial anthelmintics (96.8–97.2%), and the best results (> 75% larval mortality) were provided by the products obtained from cumin, cinnamon, coriander, and laurel.

The study of plant extracts and phytotherapy has increased due to the reduced efficacy of some commercial anthelmintics to control GIN, which may evolve into anthelmintic resistance (AR) in part due to the overuse of these drugs to maintain animal productivity [2, 3, 27]. Besides this, the search for secondary metabolites derived from essential oils is important in equine nematodes because they promote one health considering that these species

Table 3 Natural mortality (C), significance of the regression parameters and lethal concentration 50, 95 and 99 of the different plants used in the hatching test of cyathostomin eggs in horses

Scientific name	C	β_o	β_1	β_o	β_1	LC_{50} (mg/mL)	LC_{95} (mg/mL)	LC_{99} (mg/mL)
Genista tridentata	0.0714	− 6.1176	1.912	ns	ns	1583.0	11,478	26,079
Ulex europeus	0.0769	− 23.6819	8.283	ns	ns	723.0	1142	1380
Cytsus scoparius	0.0765	− 1.5835	0.805	**	**	92.7	10,238	71,906
Pinus	0.0726	− 2.1066	0.9056	**	**	211.9	13,878	78,492
Urtica urens	0.0788	− 1.9249	1.277	**	**	32.2	624	2133
Laurus nobilis	0.0756	0.2041	2.0712	**	**	0.8	5.0	10.6
Eucalyptus globosus	0.0888	− 2.238	1.3291	**	**	48.3	835	2718.0
Oxfendazol 2.5% + Closantel 5%	0.0823	− 1.242	3.0226	*	**	2.6	9.0	15.2
Piper nigrum L	0.091	− 4.5387	2.1748	**	**	122.2	697.0	1434.0
Pelargonium graveolens	0.957	− 0.7931	1.0636	**	**	5.6	196.0	857.1
Oxfendazol 2.5% + Closantel 5%	0.0407	0.8033	3.7258	**	**	0.6	1.7	2.6
Prunus lauracerasus	0.026	− 0.8455	3.2302	**	**	1.8	5.9	9.6
Prunus lauracerasus	0.23	− 1.8696	4.44	*	**	2.6	6.2	8.8
Ocimum basilicum	0.1885	− 2.5316	2.4895	**	**	10.4	47.6	51.5
Camelia japonica	0.1913	− 9.2185	4.94	**	**	73.6	158.4	217.7
Syzygium aromaticum	0.1966	− 9.07	5.0311	**	**	63.7	135.2	184.6
Illicium verum	0.0235	1.048	3.823	**	**	0.53	1.43	2.16
Chamaecyparis lawsoniana	0.03	1.2035	1.87	**	**	0.23	1.72	3.98
Cuminum cyminum	0.0396	3.44	2.69	**	**	0.05	0.22	0.39
Cinnamomum verum	0.0226	1.22	4.54	**	**	0.54	1.24	1.75
Coriandrum sativum	0.0333	1.04	3.78	**	**	0.53	1.44	2.19

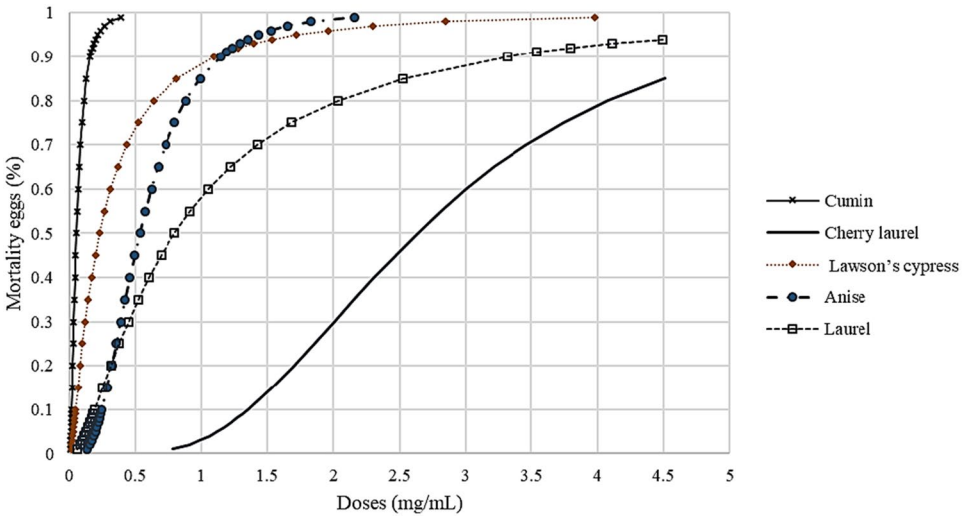

Fig. 1 Probit curve of some essential oils from plants from the region of Galicia (NW Spain)

share some pathogens, niche adaptation, epidemiology, vector transmission, culture resources, food safety and biosecurity with humans [14].

Usefulness of some plants as sustainable alternatives for the control of nematodes is based on the level of mortality caused on eggs and/or larvae, but also on the concentration required to achieve that antiparasitic effect. In the present investigation, the EOs obtained from cumin, cinnamon, coriander, star anise, Lawson's cypress, laurel, and orange peel reached egg-mortality values between 76.9% and 98.9% when using concentrations lower than 10 mg/mL. An antagonistic effect on cyathostomin eggs higher than 92% was recorded with concentrations between 31.3 and 62.5 mg/mL for basil, mallow, and clove, whereas a concentration of 250 mg/mL was needed for eucalyptus and black pepper to attain an efficacy of 98.8% and 93.3%, respectively. These results agree partly with previous research

Table 4 Concentrations at which the vapor stripping products of different plants present mortality of horse cyathostomin larvae

Date	Product	Scientific name	Concentration (mg/mL)	Mortality (%)
13/02/2023	Eucalyptus	*Eucalyptus globulus*	250	9.3
13/02/2023	Nettle	*Urtica dioica*	250	2.4
13/02/2023	Bay	*Laurus nobilis*	250	76.2
13/02/2023	Broom	*Cytsus scoparius*	250	0.3
13/02/2023	Pine	*Pinus*	250	1.5
13/02/2023	Prickly broom	*Genista tridentata*	250	5.0
13/02/2023	Gorse	*Ulex europeaus*	250	1.7
27/02/2023	Olive	*Olea europaea*	250	2.6
27/02/2023	Black pipper	*Piper nigrum*	250	2.6
27/02/2023	Silver wattle flower	*Acacia dealbata*	250	0.0
27/02/2023	Mallow	*Pelargonium graveolens*	250	2.8
27/02/2023	Holly	*Ilex aquifolium*	250	2.4
28/02/2023	Oxydrench		1.3	96.8
28/02/2023	Endoex (Closantel)		12.5	97.2
28/02/2023	Cherry laurel leaf	*Prunus lauracerasus*	63	98.0
28/02/2023	Olive	*Olea europaea*	250	63.6
28/02/2023	Yew	*Taxus baccata*	250	67.5
28/02/2023	Silver wattle leaf	*Acacia dealbata*	250	11.8
28/02/2023	Orange peel	*Citrus sinensis*	500	29.3
22/03/2023	Basil	*Ocimum basilicum*	125	1.2
22/03/2023	Camelia	*Camelia japonica*	250	2.6
22/03/2023	Clove	*Syzygium aromaticum*	250	17.6
22/03/2023	Star anise	*Illicium verum*	15.6	71.4
22/03/2023	Lawson's cypress	*Chamaecyparis lawsoniana*	250	14.2
22/03/2023	Cumin	*Cuminum cyminum*	7.8	95.1
22/03/2023	Cinnamon	*Cinnamomum verum*	15.6	87.6
22/03/2023	Coriander	*Coriandrum sativum*	250	83.2

Table 5 Natural mortality (C), significance of the regression parameters and lethal concentration 50, 95 and 99 of the different plants used in the larval mortality test of horse cyathostomins

Scientific name	C	βo	β1	βo	β1	LC_{50} (mg/mL)	LC_{95} (mg/mL)	LC_{99} (mg/mL)
Laurus nobilis	0.02	− 1.58	0.71	**	**	911.3	34,204.0	310,231.0
Oxfendazol 2.5% + Closantel 5%	0	− 2.31	2.52	ns	ns	8.2	36.9	68.7
Prunus lauracerasus	0	− 4.03	2.58	**	**	36.3	157.8	289.8
Closantel 50 mg/mL	0	− 0.47	2.69	ns	**	1.5	6.2	11.0
Illicium verum	0.02	− 5.24	5.00	**	**	11.1	23.75	32.5
Cuminum cyminum	0.03	− 2.00	4.00	**	**	3.2	6.5	8.9
Cinnamomum verum	0.02	− 3.64	4.34	**	**	6.9	16.49	23.7

conducted in Brazil, regarding low concentrations of cumin EO (0.35 to 9.4 mg/mL) provoked high rates of mortality (93% to 98%) on the ruminants gastrointestinal nematode *Haemonchus contortus* [9], and even against the eggs of the liver trematode *Fasciola hepatica* [24]. Full (100%) ovicidal effect on *H. contortus* eggs at a dose of 1.72 mg laurel EO/mL has been demonstrated [34].

Because of third-stage larvae of cyathostomins are the infective stages, the influence of plant EOs on L3 survival was also determined in the current study. The best results (95.1%) were achieved with cumin at 7.8 mg/mL, followed by 15.6 mg cinnamon EO/mL (87.6%), while 250 mg/mL were required to attain 76.2% and 88.3% L3 mortality by using EOs from laurel and coriander, respectively. It has been reported a 31% *H. contortus* larval development with cumin at 9.4 mg/mL [9], and 87.5% immobility in adult *H. contortus* with 1.72 mg laurel EO/mL [34].

EOs are the volatile liquid fractions, generally obtained by hydro-distillation, which contain the substances responsible for the flavor, odor or scent to an aromatic plant [1]. These are generally complex mixtures of up to more than 100 components that can be low molecular weight aliphatic compounds (alkanes, alcohols, aldehydes, ketones, esters, and acids), monoterpenes, sesquiterpenes, and phenylpropanes [12]. Different EOs tested in many studies against bacteria and fungi provided promising results, and against external parasites such as bovine ticks also [30]. Nevertheless, there is scarce information about their effect on nematode eggs and larvae, and most of tests have been focused on *H. contortus* due to its great importance in sheep production [10, 34, 37]. Data collected in the present investigation showed that there are essential oils from plants able to reduce the survival of eggs and/or larvae of horse cyathostomins. Besides this, some liquid (non EO) extracts with anthelmintic activity were obtained in certain plants. In concrete, percentages of egg-mortality around 92.7% and 96.8% were obtained with cherry laurel (3.9 mg/mL) and yew (250 mg/mL), respectively. In relation to L3 mortality, the best results (98%) were reached by using a concentration of 63 mg cherry laurel/mL. Previous studies demonstrated that aqueous crude extracts from 37 species of Australian native plants provided anthelmintic activity in vitro against cyathostomin larvae [29]. Other investigations also addressed different types of extracts [8, 15], but the search for parasiticidal active compounds continues [6].

An interesting question concerns the EO yielding, because this might influence the possibilities of obtaining sufficient quantities and thus its utilization. In the present study, the best results were obtained from seeds of clover and black pepper, and the lowest with basil leaves. By taking into account together EO yielding, the egg-mortality values, and the concentrations required to achieved them, the most promising source of EOs with ovicidal activity appear the leaves of eucalyptus, then basil, mallow, black pepper, orange peel, clover and laurel. When considering EO yielding together with the activity against L3 cyathostomins and the concentration needed, the efficiency reduced, with laurel leaves, coriander seeds and cinnamon stem offering the best results. It has been demonstrated that the crude aqueous extract of cinnamon mixed with oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*) and chili (*Capsicum annuum*) provided high effectiveness against *H. contortus* eggs in sheep [40].

Finally, the easiness to get the plants appear essential for the success of this approach. Accordingly, local plants should be considered due to their availability ensures the production of enough quantities [7]. Some of the EOs tested in the present study are obtained from seeds of foreign plants commonly used as condiments stood out (black pepper, basil, coriander, clove, cumin, star anise or cinnamon), and others belong to gardening shrubs or tress (mallow, basil, Lawson´s cypress), whereas eucalyptus and laurel are frequently cultured in NW Spain due to their adaptability. In this area, orange peel is not easy to obtain.

5 Conclusions

The preliminary study of different plants collected in NW Spain revealed the presence of certain species providing essential oils with parasiticide activity on cyathostomins, gastrointestinal nematodes affecting horses worldwide. The EOs obtained from basil, mallow, and clove have elevated ovicidal activity at low concentrations, and cumin and cinnamon against cyathostomin larvae. Laurel EO exerts an important antagonistic effect on both eggs and larvae, then appears a very firm candidate to develop strategies for their control in equines based also on the concentration to reach the parasiticide effect and the easiness to produce enough quantities. Further investigations are required to determine the appropriate formulation, together with the practical dosage, and to discard the possibility of side effects.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate Not applicable.

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