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In Vitro Activity of the Lamiaceae Family Species on *Ancylostoma* spp. Eggs

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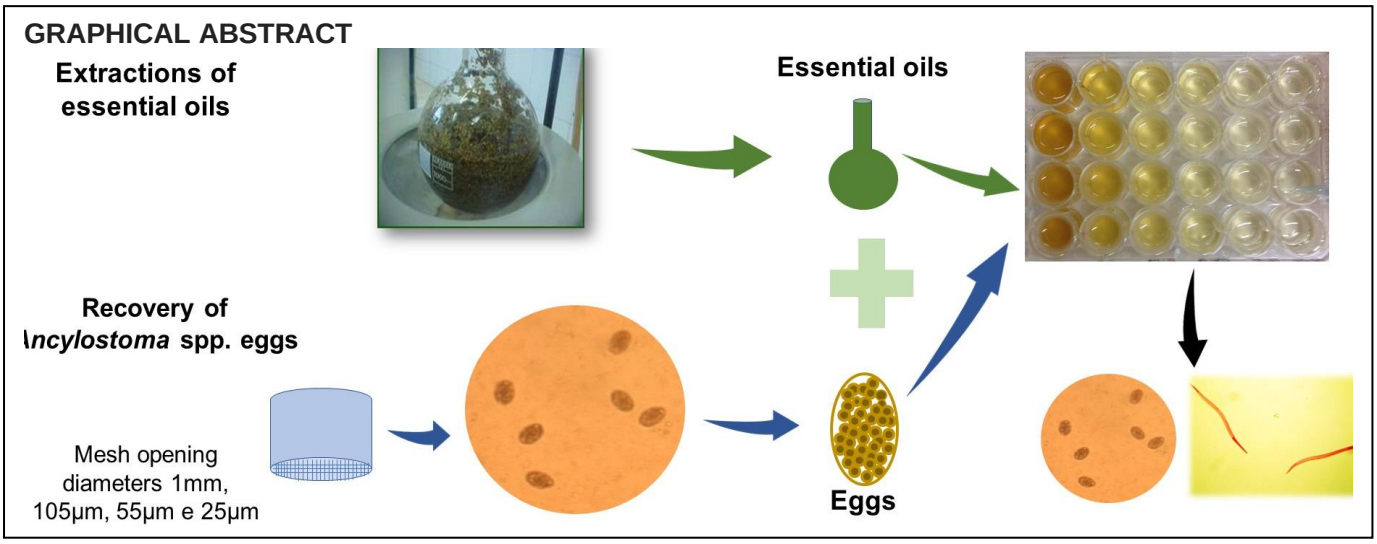
HIGHLIGHTS

- Medicinal plants in the control of hookworm eggs.
- Lamiaceae family species showed inhibition of *Ancylostoma* spp. eggs.
- Herbal essential oil blocking the *Ancylostoma* spp. cycle.
- Phytotherapy in the control of zoonoses.

Abstract: The in vitro anthelmintic activity of essential oils from the Lamiaceae family species against *Ancylostoma* spp. eggs was evaluated, as well as the chemical composition by GC/MS of these essential oils. The major chemical compounds for *O. vulgare* were 4-terpineol, while for the essential oil of *O. majorana*, o 4-terpineol, and for the oil of *R. officinalis* it was observed cineole as major components. For this, hatchability tests were performed, where the parasite eggs were exposed to concentrations of 0.07% to 2.5% of the essential oils of *Origanum vulgare*, *Origanum majorana* and *Rosmarinus officinalis*. The assay was accompanied by a control with thiabendazole hydrochloride, a control with distilled water and a control with tween 80. The chemical composition of each essential oil sample was determined by gas chromatography. It was observed that all oils showed ovicidal action, and the percentage of inhibition of hatchability of *R. officinalis* oil was greater than 90% at concentrations from 0.62% to 2.5%. As for the essential oils of *O. vulgare* and *O. majorana*, the concentrations of 0.31% to 2.5% were those that presented an inhibitory percentage greater than 90%. Thus, this study showed that the essential oils of *R. officinalis*, *O. vulgare* and *O. majorana* inhibit the hatchability of *Ancylostoma* spp., being promising for the control of this helminth.

Keywords: Alternative control; helminths; herbal medicines.



INTRODUCTION

The interaction of man with pets, although healthy, requires care in relation to the health and well-being of both, especially with regard to zoonotic diseases, especially parasitic ones. Dogs and cats are currently part of the family and have an intimate relationship with its members. This close relationship exposes people to various infectious agents that are eventually transmitted through contact with the feces of these animals. In this scenario, companion animal endoparasites deserve special attention due to their high prevalence, being *Ancylostoma* spp. with high occurrence in dogs and whose importance in public health is indisputable [1, 2].

Hookworms are helminth nematodes belonging to the Ancylostomatidae family. Most species parasitize the small intestine, feeding on blood from definitive hosts. In canines, *Ancylostoma caninum* and *Ancylostoma braziliense* are the main etiological agents of hookworm, an intestinal parasitosis that affects young animals up to one year old and puppies, due to infections via transmammary route and the immaturity of the canine immune system [3].

Ancylostoma spp. has a wide geographic distribution and high prevalence in the environment where people and dogs coexist [4, 5], causing a risk to the health of these individuals. The main source of human contamination is public environments, such as squares, parks and beaches, where parasitized dogs defecate, eliminating the eggs that contaminate the soil, which can lead to the occurrence of Cutaneous Larva Migrans, a dermatozoonosis of importance for public health [6]. Associated with the high rate of parasitism, there are difficulties in relation to the treatment of infected animals, due to the adaptive phenomenon of resistance to anthelmintics [7, 8, 9, 10]. According to some studies, the frequency of use of antiparasitic compounds and/or the rapid alternation between them are the main factors responsible for triggering the selection process in a population of parasites [11].

Given this reality, research with medicinal plants has been encouraged, in search of new molecules and new treatment methods, highlighting the use of essential oils. These molecules are very promising, as Brazil has one of the richest flore in the world, with more than 56,000 species of plants [12]. Thus, several plants popularly used have been cited and indicated for the control and treatment of parasitosis, with emphasis on the plants of the Lamiaceae family. The objectives of the study were to verify the in vitro anthelmintic activity of essential oils of *Origanum vulgare*, *Origanum majorana* and *Rosmarinus officinalis* on eggs of *Ancylostoma* spp. of dogs and determine their major chemical constituents.

MATERIAL AND METHODS

Collection and storage of fecal samples

The fecal samples were obtained through environmental collection of feces from adult dogs not dewormed, from the Municipal Kennel of Pelotas, Veterinary Clinic and Veterinary Clinic Hospital - Favet/UFPel. The samples were stored in plastic bags, properly identified, cooled and sent to the Parasitology Laboratory to be processed immediately.

Experimental design

The experiment was conducted following a completely randomized design, in a two-factor arrangement with four replications, where the first factor was "oil source" (rosemary; marjoram; oregano) and the second factor "oil concentration" (0.07%; 0.15%; 0.31%; 0.62%; 1.25%; 2.5%). The variable evaluated was the percentage of inhibition of the hatchability of *Ancylostoma* spp.

Hatchability tests were performed in 24-well microculture plates, where six concentrations (0.07% to 2.5) of the essential oils of *Origanum vulgare*, *Origanum majorana* and *Rosmarinus officinalis* were distributed along with a suspension containing approximately 150 eggs of the parasite. In addition, a test with mineral oil grade p.a. and with tween 80, which was used to emulsify the essential oil, at the same dilutions, was used as a control for essential oils, in order to evaluate the mechanical action of the oils on the parasite's eggs.

Thiabendazole hydrochloride (0.025 mg/mL), a negative control with distilled water. All concentrations were tested in quadruplicate and the plates were sealed with plastic film and incubated at 28°C with 80% relative humidity for 36hrs. The reading of the plates was performed with the aid of an inverted light microscope [13].

Recovery of *Ancylostoma* spp. eggs

Feces samples were processed using the Willis Mollay flotation technique [14] to determine the positivity of the samples for eggs of *Ancylostoma* spp. For egg recovery, positive feces were processed according to the technique described by Hubert and Kerboeuf [15], with modifications. First, the feces were macerated and added with water at a temperature close to 40°C, followed by the passage of feces in four sieves with the following mesh opening diameters: 1 mm, 105 micrometers, 55 micrometers and 25 micrometers. Eggs retained in the last sieve were collected by washing with distilled water. This material obtained was transferred to 50mL tubes and centrifuged at 3,000 rpm at room temperature for five minutes. The supernatant was discarded and the pellet resuspended in saturated saline solution and centrifuged again for five minutes. After the last centrifugation, the supernatant was passed again in 25-micron sieve, followed by washing and quantifying the eggs.

Extractions and chromatographic analysis of essential oils

Plants of the species *O. vulgare*, *O. majorana* and *R. officinalis* were acquired from a commercial distributor (Luar Sul®), with quality and origin certification for the extraction of essential oils, which were carried out at the Natural Products Research Laboratory from the Department of Organic Chemistry at the Federal University of Pelotas. The oil extraction technique was the same for the three plants following the guidelines of the Brazilian Pharmacopoeia IV [16]. The technique for obtaining the oils consisted of weighing 100g of the dry leaves and submitting them to extraction with steam drag, in a Clevenger-type apparatus, for 4 hours, being carried out in triplicate. After extraction, the oil obtained was dried with anhydrous sodium sulfate, stored in an amber flask and kept under refrigeration until its use.

The identification of essential oil compounds was performed using a gas chromatograph coupled to a mass detector, model GC/MS-QP 2010SE (Shimadzu, Japan), equipped with an AOC-20i auto-injector. The separation took place in a capillary column RTX-5MS (Restek, USA), with dimensions of 30 m x 0.25 mm x 0.25 µm, under the following chromatographic conditions: initial temperature of 40°C, rising to 10°C/min, up to 280ºC, remaining at this temperature for 10 min; injected volume, 1 µL; interface, 300ºC; injector temperature, 280°C; carrier gas, helium; linear gas flow, 1.22 mL.min-1; Split, 1:50; run in scan mode; mass range, 40 to 700 *m/z* and filament voltage, 70eV. Quantifications were made by standardized area and compound identification by mass spectrometer, using the NIST 8 library of the GC/MS. Oil samples were diluted in hexane (analytical grade, ultrapure).

Expressions of Results

The results were expressed as the mean percentage of inhibition of the hatchability of the quadruplicate, with the efficacy of each treatment determined according to the Equation: IH= L/(L+V) x 100. Where, “IH” is the percentage of inhibition of the hatchability, “L” is the number of hatched larvae, and “V” is the number of eggs.

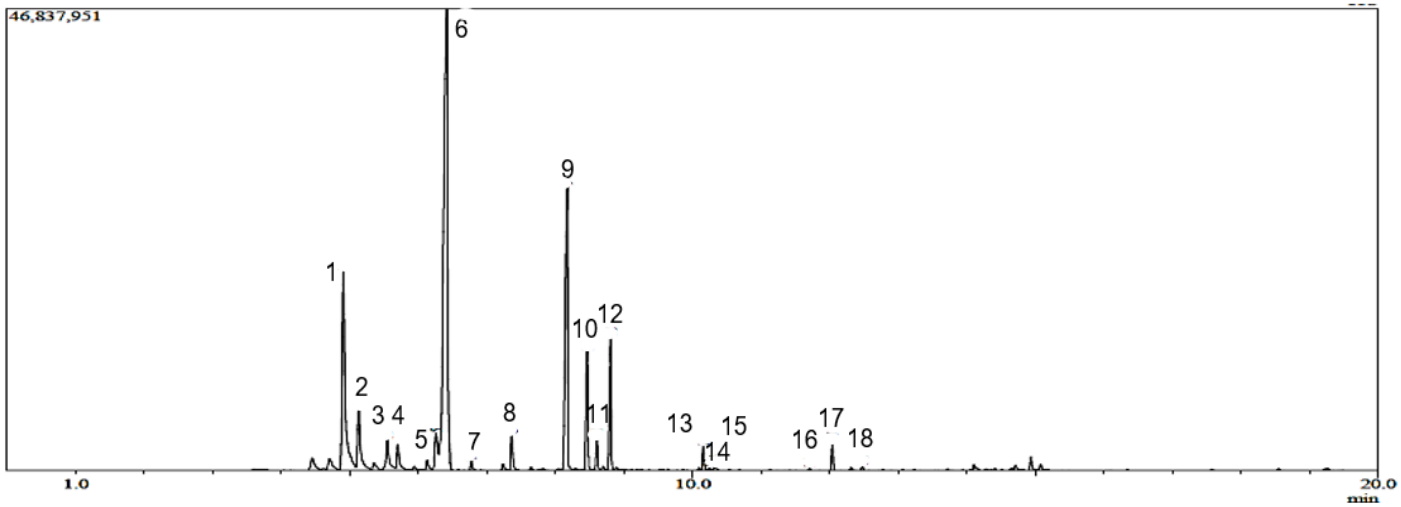
Statistical analysis

Outliers were identified by plotting externally studentized residuals (*RStudent*) versus predicted values (Y variable) and also by the Cook's Distance graph. From *RStudent*, values that were outside the range [-2; 2] were considered outliers and their corresponding observations were removed from the database [17]. The data obtained were analyzed for normality using the Shapiro-Wilk test, for homoscedasticity using the Hartley test, and the independence of the residues was graphically verified.

Subsequently, the data were submitted to analysis of variance by the F test ($p \leq 0.05$). After detecting significance for the quantitative factor (concentration), regression analysis was performed using adjustment to a polynomial mathematical model, as shown in Equation $y = a\ (1 - e^{-bx})$, in which: "y" is the percentage of inhibition of egg hatching, "a" and "b" are the constants of the equation and "x" the oil concentration factor (%). Model selection was based on the following parameters: (a) low residual values; (b) low p value; (c) low standard deviation; and (d) R^2 high and R^2 corrected.

RESULTS

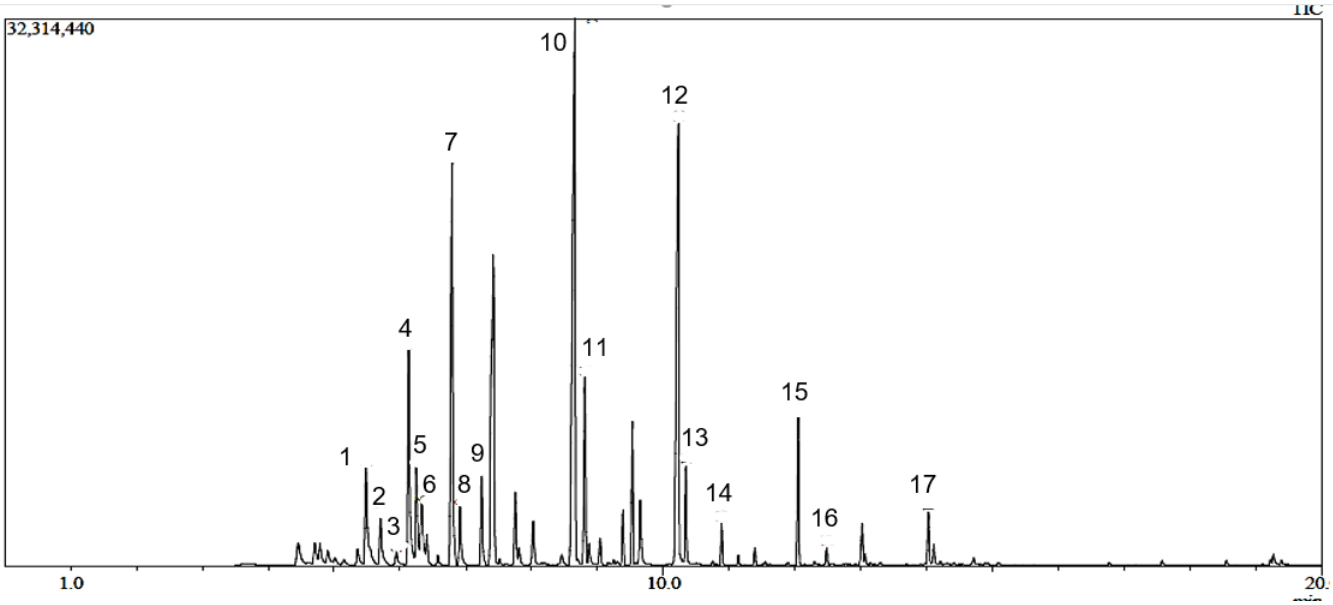
The gas chromatography GC/MS of the essential oil of *R. officinalis* showed the presence of a total 18 compounds (Figure 1), and the main constituents identified were cineole (42.12%), camphor (16.37%) and alpha-Pinene (14, 76%), while for the sample of *O. vulgare* there were total 17 components (Figure 2), among which Terpinen-*4*-ol (27.24%), thymol (19.78%) and gamma-Terpinene (14.23%) were the majority. For *O. majorana* oil, a total 14 components were identified (Figure 3), highlighting Terpinen-*4*-ol (35.99%), gamma-Terpinene (16.76%) and alpha-Terpinene (11.43%), as shown in Table 1.



Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Name
1	4.906	62490844	14.76	20213721	alpha.-Pinene $$ Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl- $$ 2-Pinene $$ 2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene $$ Pinene,
2	5.129	16398410	3.87	6183536	Camphene $$ Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene- $$ 2,2-Dimethyl-3-methylenebicyclo[2.2.1]heptane
3	5.546	8797162	2.08	3374412	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)- $$ 2(10)-Pinene, (1S,5S)-(-)- $$ (-)-.beta.-Pinene $$ (-)-2(10)-Pinen
4	5.701	5852719	1.38	2803891	.beta.-Myrcene $$ 1,6-Octadiene, 7-methyl-3-methylene- $$ Myrcene $$ 7-Methyl-3-methylene-1,6-octadiene $$ 7-Methyl-3-m
5	6.257	11373752	2.69	3679725	o-Cymene $$ Benzene, 1-methyl-2-(1-methylethyl)- $$ o-Cymol $$ o-Isopropyltoluene $$ 1-Isopropyl-2-methylbenzene
6	6.418	178238887	42.11	46158181	Eucalyptol $$ Cineole $$ 2-Oxabicyclo[2.2.2]octane, 1,3,3-trimethyl- $$ p-Menthane, 1,8-epoxy- $$ p-Cineole $$ Cajeputol
7	6.773	1565746	0.37	897147	gamma.-Terpinene $$ 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- $$ .gamma.-Terpinen $$ p-Mentha-1,4-diene
8	7.363	7205055	1.70	3704729	1,6-Octadien-3-ol, 3,7-dimethyl- $$ .beta.-Linalool $$ Linalol $$ Linalool $$ Linalyl alcohol $$ 2,6-Dimethyl-2,7-octadien-6-o
9	8.171	69275956	16.37	28805482	(+)-2-Bornanone $$ Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)- $$ Camphor, (1R,4R)-(+)- $$ Alcanfor $$ d-2-Bornanc
10	8.463	24272875	5.73	11992013	Isoborneol $$ Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, exo- $$ exo-2-Hydroxy-1,7,7-trimethylnorbornane $$ Isobornyl alco
11	8.603	5220837	1.23	3199960	Terpinen-4-ol $$ 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- $$ p-Menth-1-en-4-ol $$ 1-Terpinen-4-ol
12	8.806	22867523	5.40	13429342	alpha.-Terpineol $$ 3-Cyclohexene-1-methanol, .alpha.,.alpha.4-trimethyl- $$ p-Menth-1-en-8-ol $$ Terpineol schlechthin $$ Te
13	10.158	3537090	0.84	2346385	Bornyl acetate $$ Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, endo- $$ Borneol, acetate $$ Bornyl acetic ether
14	10.247	228682	0.05	140712	Thymol $$ Phenol, 5-methyl-2-(1-methylethyl)- $$ p-Cymen-3-ol $$ Thyme camphor $$ 2-Isopropyl-5-methylphenol
15	10.327	784992	0.19	479419	Phenol, 2-methyl-5-(1-methylethyl)- $$ Carvacrol $$ p-Cymen-2-ol $$ Antioxine $$ Isothymol $$ Karvakrol $$ 2-Hydroxy-p-c
16	11.709	302521	0.07	197781	Methyleugenol $$ Benzene, 1,2-dimethoxy-4-(2-propenyl)- $$ Benzene, 4-allyl-1,2-dimethoxy- $$ Ent 21040 $$ Eugenol meth
17	12.041	4244778	1.00	2897591	Caryophyllene $$ Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4E,9S*)]-
18	12.480	630506	0.15	393714	Humulene $$ .alpha.-Caryophyllene $$ 1,4,8-Cycloundecatriene, 2,6,6,9-tetramethyl-, (E,E,E)- $$ .alpha.-Humulene
		423288335	100.00	150897741	

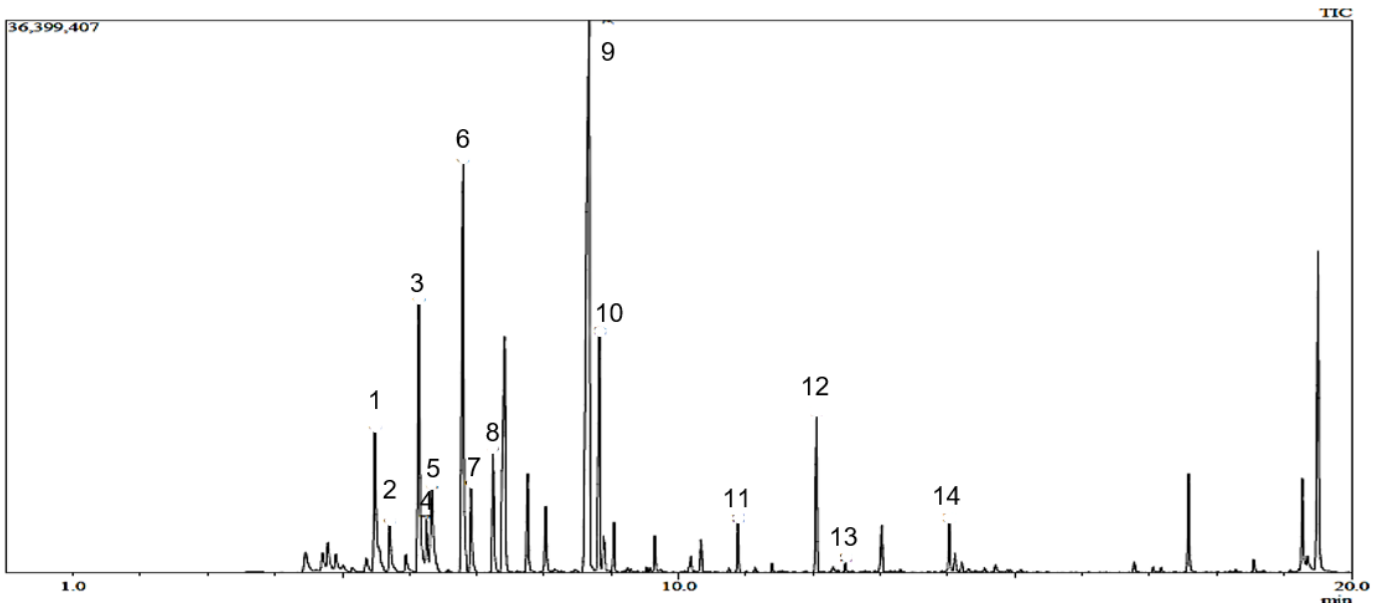
Figure 1. Chromatogram figure of *Rosmarinus officinalis* with its 18 components peaks.



Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Name
1	5.483	13699853	4.01	5680209	Cyclohexene, 4-methylene-1-(1-methylethyl)- $$ p-Mentha-1(7),3-diene $$ .beta.-Terpinen $$ .beta.-Terpinene
2	5.703	7018913	2.06	2954823	.beta.-Myrcene $$ 1,6-Octadiene, 7-methyl-3-methylene- $$ Myrcene $$ 7-Methyl-3-methylene-1,6-octadiene
3	5.946	1580867	0.46	711817	alpha.-Phellandrene $$ 1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)- $$ .alpha.-Fellandrene
4	6.134	27815020	8.15	12675753	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- $$ .alpha.-Terpinene $$ .alpha.-Terpinen
5	6.251	9534246	2.79	5374921	o-Cymene $$ Benzene, 1-methyl-2-(1-methylethyl)- $$ o-Cymol $$ o-Isopropyltoluene
6	6.332	7192710	2.11	3015456	beta.-Phellandrene $$ Cyclohexene, 3-methylene-6-(1-methylethyl)- $$ p-Mentha-1(7),2-diene $$ Phellandrene, .beta
7	6.790	48597776	14.23	23824413	gamma.-Terpinene $$ 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- $$ .gamma.-Terpinen
8	6.913	7127659	2.09	3459751	trans-Sabinene hydrate (trans for Me vs IP) $$ trans-Sabinene hydrate $$ trans-Sabinene hydroxide
9	7.240	9376102	2.75	5286180	-Carene $$ Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl- $$ .delta.-2-Carene $$ (.+/-.)-2-Carene
10	8.655	93058583	27.25	32218905	Terpinen-4-ol $$ 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- $$ p-Menth-1-en-4-ol
11	8.809	17841009	5.22	11040598	alpha.-Terpineol $$ 3-Cyclohexene-1-methanol, .alpha.,.alpha.4-trimethyl- $$ p-Menth-1-en-8-ol
12	10.230	67529786	19.78	25968073	Thymol $$ Phenol, 5-methyl-2-(1-methylethyl)- $$ p-Cymen-3-ol $$ Thyme camphor
13	10.344	8226633	2.41	5698359	Phenol, 2-methyl-5-(1-methylethyl)- $$ Carvacrol $$ p-Cymen-2-ol
14	10.885	3540424	1.04	2413076	gamma.-Elemene $$ 1-Methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-1-vinylcyclohexane), (1R-trans)
15	12.049	13310553	3.90	8690478	Caryophyllene $$ Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4E,9S*)]-
16	12.483	1340337	0.39	950385	Humulene $$ .alpha.-Caryophyllene $$ 1,4,8-Cycloundecatriene, 2,6,6,9-tetramethyl-, (E,E,E)- $$ .alpha.-Humulene
17	14.025	4672970	1.37	3049184	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]- $$
		341463441	100.00	153012381	

Figure 2. Chromatogram figure of *Origanum vulgare* with its 17 components peaks.



Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Name
1	5.487	22361850	6.42	9279569	p-Mentha-1(7),3-diene $$ .beta.-Terpinen $$ .beta.-Terpinene $$ Beta terpinene
2	5.707	6589943	1.89	3138610	beta.-Myrcene $$ 1,6-Octadiene, 7-methyl-3-methylene- $$ Myrcene
3	6.140	39834386	11.43	17824662	alpha.-Terpinene $$ .alpha.-Terpinen $$ p-Mentha-1,3-diene $$ Terpilene
4	6.253	4616986	1.33	2808347	o-Cymene $$ Benzene, 1-methyl-2-(1-methylethyl)- $$ o-Cymol $$ o-Isopropyltoluene
5	6.338	15017422	4.31	5209790	beta.-Phellandrene $$ Cyclohexene, 3-methylene-6-(1-methylethyl)- $$ p-Mentha-1(7),2-diene
6	6.797	58407410	16.76	26935300	gamma.-Terpinene $$ 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- $$ .gamma.-Terpinen
7	6.917	9061470	2.60	5421240	trans-Sabinene hydrate (trans for Me vs IP) $$ trans-Sabinene hydrate $$ trans-Sabinene hydroxide
8	7.243	14126615	4.05	7908344	2-Carene $$ Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl- $$ .delta.-2-Carene
9	8.671	125383155	35.98	36335216	Terpinen-4-ol $$ 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- $$ p-Menth-1-en-4-ol $$ 1-Terpinen-4-ol
10	8.822	26774129	7.68	15440023	alpha.-Terpineol $$ 3-Cyclohexene-1-methanol, .alpha.,.alpha.4-trimethyl-
11	10.884	4632295	1.33	3249382	.gamma.-Elemene $$ 1-Methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-1-vinylcyclohexane), (1R-trans)-
12	12.050	15915879	4.57	10237277	Caryophyllene $$ Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4E,9S*)]-
13	12.482	920057	0.26	612882	Humulene $$ .alpha.-Caryophyllene $$ 1,4,8-Cycloundecatriene, 2,6,6,9-tetramethyl-, (E,E,E)-
14	14.025	4803310	1.38	3155978	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha
		348444907	100.00	147556620	

Figure 3. Chromatogram figure of *Origanum majorana* with its 14 components peaks.

Table 1. Chemical characterization of *Rosmarinus officinalis*, *Origanum vulgare* and *Origanum majorana* essential oils with the respective percentages and retention time determined by gas chromatography.

Peak	Retention time	Compound	*O. vulgare* concentration	*O. majorana* concentration	*R. officinalis* concentration
1	5.483	β-Terpinene	4.01	6.42	ND
2	5.703	β-Myrcene	2.06	1.89	1,38
3	5.546	β-Pinene	ND	ND	2.08
4	5.946	α-Phellandrene	0.46	ND	ND
5	6.134	α-Terpinene	8.15	11.43	ND
6	4.906	α-Pinene	ND	ND	14.76
7	5.129	Camphene	ND	ND	3.87
8	6.418	Cineole	ND	ND	42.12
9	6.251	Cimene	2.79	1.33	2,69
10	6.332	β-Phellandrene	2.11	4.31	ND
11	6.790	Gamma-Terpinene	14.23	16.76	0,37
12	6.913	Trans-Sabinene hydrate	2.09	2.60	ND
13	7.240	Delta-2-carene	2.75	4.05	ND
14	7.363	Linalool	ND	ND	1.70
15	8.171	Camphor	ND	ND	16.37
16	8.463	Isoborneol	ND	ND	5.73
17	8.655	Terpinen-4-ol	27.24	35.99	1,23
18	8.809	α-Terpineol	5.22	7.68	5,40
19	10.158	Bonyl acetate	ND	ND	0.84
20	10.230	Thymol	19.78	ND	0.05
21	10.344	Carvacrol	2.41	ND	0.19
22	10.885	Gama-terpineno	1.04	1.33	ND
23	11.709	Methyl Eugenol	ND	ND	0.07
24	12.049	Caryophyllene	3.90	4.57	1.00
25	12.483	Humulene	0.39	0.26	0.15
26	14.025	Spathulenol	1.37	1.38	ND

ND: Not detected

The *in vitro* test to evaluate the percentage of inhibition of egg hatchability showed that the essential oil samples, from the three plants under study, presented anthelmintic action on *Ancylostoma* spp. eggs, in increasing percentages, according to the increment at the concentrations tested, as shown in Figure 4.

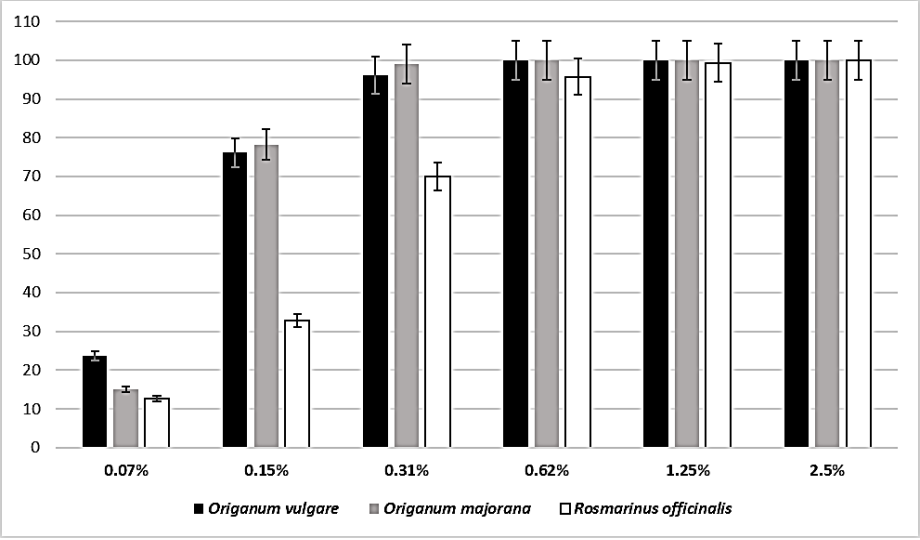


Figure 4. Results of the hatchability inhibition tests of essential oils of *O. vulgare*, *O. majorana* and *R. officinalis* at concentrations from 0.07 to 2.5%.

The positive control showed 100% inhibition of hatchability, showing egg susceptibility, while the negative control and tween 80 showed an average hatchability percentage of 97.73% and 93%, respectively, demonstrating good egg viability.

In the test performed with mineral oil at concentrations from 0.07% to 2.5%, it was possible to observe that eggs hatched more than 98.57%. This evaluation became important, as the mineral oil did not prevent the hatchability of *Ancylostoma* spp., demonstrating that the activity of essential oils of the Lamiaceae family was not due to the direct mechanical action that the oil could be exerting on the surface of the eggs, but probably due to the presence of bioactive compounds that interfered with this hatchability.

In all hatchability tests, with the different oils, increasing percentages of hatchability inhibition were observed according to the increase in oil concentration. For oregano at a concentration of 0.07%, the inhibition was 23.60% of hatchability, while at concentrations of 0.62% to 2.5%, the percentage was 100%. At a concentration of 0.15%, the inhibition of hatchability remained above 50%, while the concentration of 0.31% showed a percentage of inhibition above 90%.

The test result for marjoram showed an inhibitory percentage of 100% at concentrations from 2.5% to 0.62%, being above 90% at a concentration of 0.31% and above 50% at a concentration of 0.15 %, and close to 15% at the concentration of 0.07%.

The inhibition percentages for rosemary ranged from 12.62% to 100%, at concentrations from 0.07% to 2.5%, respectively. The 0.62% and 1.25% concentrations were more than 90% effective, while the 0.31% concentration was above 50% and the 0.15% was below this percentage, as shown in image 1.

Regarding the comparison of the activity of oils from different genera of the Lamiaceae family, it was observed that *Rosmarinus* spp. showed a lower performance than *Origanum* spp., which for the concentration of 0.31% for the oils of oregano and marjoram the percentage of inhibition of hatchability was 96.16% and 99%, respectively, while for rosemary, at the same concentration, the percentage of inhibition was 69.98%. The same occurred with the concentration of 0.15%, which for oregano and marjoram showed inhibition greater than 50% (76.11% and 78.24%), respectively, while for rosemary the percentage was below 50%.

DISCUSSION

The variations observed in the analysis of essential oils were already expected, since there are many factors that can interfere in the chemical composition of a plant's essential oils, such as: harvest time; degree of humidity; part of the plant used; drying temperature; extraction methods and type of planting. The different species of the Lamiaceae family produce many secondary metabolites, which generally have a complex chemical structure, determining the diversity of bioactive compounds resulting in numerous biological activities [18].

Studies of different species of the Lamiaceae family have shown variations in secondary metabolites, so we can have samples rich in phenolic compounds or with a higher percentage of monoterpenic alcohols, such as hydrated cis-sabinene and terpinen-4-ol. However, in general, the major components of *Origanum majorana* are y-terpinene and terpinen-4-ol [19], confirming the result obtained by the chromatographic analysis of the marjoram oil sample in this study.

In relation to *Origanum vulgare*, several authors have identified phenols as the main compounds, especially carvacrol, thymol, terpinen-4-ol, gamma-terpene and p-cymene, being mentioned that these can reach between 80.2% to 98 % of the total oil composition of *O. vulgare* [20]. Borges and coauthors [19] demonstrated that the composition of oil of oregano has a higher content of terpinen-4-ol, thymol and carvacrol in relation to gamma-terpinene, when the plant is dried. Another study showed that the extraction of oils using flowering plants are responsible for higher levels of terpinen-4-ol, with *O. vulgare* being the major component determined by study, the terpinen-4-ol terpineol [19,21]. The results of the cited works confirm the result found in the present study, where the major component was terpinen-4-ol followed by thymol, but they differ from the results of Busatta [22] who showed lower percentages of gamma-terpinene (12.32%) and terpinen-4-ol (21.43%), this difference probably occurred due to the origin of the plant.

According to Silva and coauthors. [23], the main components of rosemary essential oil are 1.8-cineole, alpha-pinene, borneol and camphor in variable proportions depending on the origin and vegetative state of the plant. We can also find other substances in smaller proportion such as tannins, saponins, alkaloids and flavonoids.

Macedo and coauthors [24] evaluated the ovicidal and larvicidal effect of *Eucalyptus globulus* essential oil on eggs and larvae of *Haemonchus contortus*. The maximum effectiveness of the oil in eggs was 99.3% at a concentration of 21.75 mg/ml-1, while for larvae it was 98.7% at a concentration of 43.5 mg/ml-1. The chemical analysis of the oil identified the 1.8-cineole monoterpene as the main component, which is probably responsible, together with the other components for the ovicidal and larvicidal action, as it is highly

hydrophobic, acting on the cell membrane, causing damage to the cells [25]. Therefore, the oil is a good alternative for the control of gastrointestinal nematodes in sheep and goats.

Houghton and coauthors [26] described that even through data from the analysis of chemical constituents of essential oils it is not possible to state that the major component is responsible for the biological activity under study. Thus, the effect can be attributed to a constituent in a smaller proportion or to a synergism between the existing compounds in the extract.

The performance of essential oils in relation to the inhibition of hatchability, within the genus *Origanum* was very close, with 100% inhibition for concentrations from 2.5% to 0.62%, and above 90% for a concentration of 0.31%. These inhibition percentages were considered promising, because according to the classification of the efficiency index proposed by the World Association for the Advancement of Veterinary Parasitology (WAAVP), a product would be effective when it promoted above 90% of anthelmintic action; moderately effective when acting between 80 to 90%; little effective when the action was between 60 and 80% and not effective at levels below 60% [27].

The essential oils tested demonstrated anthelmintic action on the eggs of *Ancylostoma* spp. inhibiting its hatchability, however, did not prevent the blastomeration of eggs with the formation of the first instar larvae at all concentrations tested. The action of the oil on the cuticle of the parasite will determine changes in the physiology of the first instar larva, preventing it from performing ecdysis to form the infecting larva, thereby interrupting the cycle of *Ancylostoma* spp. which could in this way contribute to the environmental control of the parasite.

The activity demonstrated by the oil samples can be explained by the presence of terpinen-4-ol, which is a terpene alcohol, with polar hydroxyls and capable of making hydrogen bonds. It has been described that the compound terpinen-4-ol acts by inducing deformations in the cell membranes of microorganisms, consequently altering their permeability, which could have occurred in the case of *Ancylostoma* spp. eggs, favoring the activity for the oils of *O. vulgare* and *O. majorana*, since studies have already demonstrated its larvicidal action [28].

Some authors have reported that the presence of flavonoids and tannins may be responsible for the anthelmintic activity of some plants [29, 30]. Gardiano and coauthors [31], when evaluating the anthelmintic potential of the aqueous extract of *O. vulgare* (100 mg/mL) on the phytonematode *Rotylenchulus reniformis*, observed a reduction in egg hatching of only 28%. However, Castro and coauthors [32] demonstrated that the different extracts of *O. vulgare* inhibited the hatchability of gastrointestinal nematodes in bovines, with an inhibition percentage ranging from 8.8 to 100%, being the dye and the hydroalcoholic extract the most promising ways.

De Castro and coauthors [33] evaluated the ovicidal action of the aqueous extract of *O. basilicum*, popularly known as sweet basil, against gastrointestinal nematodes of sheep at concentrations of 40%, 20%, 10%, 5%, 2.5% and 1,25%. The in vitro test showed that the extract was efficient at the two highest concentrations, 40% and 20%, demonstrating the percentage of 100% effectiveness at concentrations of 10% and 5% the percentage was above 50%. In the present work, the inhibition of hatchability was obtained with lower concentrations in the three samples of essential oil, showing the superiority of the oil in relation to the aqueous extract [34].

The essential oil of *R. officinalis* was also tested against eggs of gastrointestinal nematodes of sheep, with 100% inhibition of hatchability at a concentration of 28.4mg/mL [35]. The concentration used to obtain results was much higher than in the present study, confirming the need for higher concentrations for this compound, which in this study showed 100% inhibition of hatchability only at its highest concentration.

The hydroalcoholic extracts of ten plants collected in Paraíba Valley region were evaluated for their ovicidal and larvicidal potential against *Ancylostoma* spp. in the canine species. In the ovicide test, none of the plant extracts showed activity against the parasite in the evaluated dilutions, however in the preliminary larvicidal activity test it was observed that among the ten species evaluated, *Nerium oleander*, *Allamanda cathartica*, *Mirabilis jalapa* and *Brugmansia suaveolens* showed larvicidal activity in the dilutions of 50mg/ml, 25mg/ml and 12.5mg/ml, inducing mortality in 100% of the larvae. Although using the same parasite as a model, the results of this research differ from the results found in the present study. The possible reasons for a better result of this study in the inhibition of hatchability are the type of extraction of the compounds and the family of plants involved [36].

CONCLUSION

The essential oils of *O. vulgare, O. majorana and R. officinalis* showed larvicidal action at concentrations of 2.5% to 0.07% on *Ancylostoma* spp. which may be a promising alternative for the control of this parasitosis, thus reducing environmental contamination and preserving human and animal health.

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