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Chemical

Essential oils against gastrointestinal nematodes in sheep in vitro and chemical composition of those plants

Óleos essenciais frente a nematódeos gastrointestinais em ovinos e a composição química dessas plantas

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ABSTRACT

The present study aimed to evaluate the *in vitro* potential of aromatic plants *Aloysia triphylla* (cidró ou erva-luísa), *Corymbia citriodora* (eucalipto-limão), *Lippia alba* (falsa-melissa) e *Piper gaudichaudianum* (pariparoba), in helminths eggs and larvae, determine their main constituents. For this, the essential oils (OEs) of the plants were extracted from leafs, in the clevenger apparatus and to determine the main constituents was carried out the gas chromatography coupled to mass spectrometry. The Egg eclodibility assay (EEA) and Larval development test (DLT) were performed using essential oils at concentrations ranged from 75 at 0.2%. mg.mL, and all tests followed by commercial anthelmintic positive control and negative control distilled water. For EEA, *A. triphylla* e *L. alba* OEs demonstred equivalente efficacy to the commercial drug at all concentrations that was tested, while *P. gaudichaudianum* OE demonstred equivalente efficacy at 15 mg/mL and the *C. citriodora* OE at 10 mg/mL. About DLT, all concentratios of the studied essential oils were effective as the comercial drug results in this test. The dominant constituent in OEs were: geraniale (*A. triphylla*), citronellal (*L. alba*), linalool (*L. alba*) and α-humuleno (*P. gaudichaudianum*). Based on the results presented here, the OEs present potential for use as natural anthelmintics for the treatment of endoparasitosis in sheep.

Keywords: Anthelmintic; Sheep production; Worms

RESUMO

O presente trabalho buscou avaliar a ação in vitro dos óleos essenciais (OEs) de *Aloysia triphylla* (cidró ou erva-luísa), *Corymbia citriodora* (eucalipto-limão), *Lippia alba* (falsa-melissa) e *Piper gaudichaudianum* (pariparoba). Os OEs foram extraídos de folhas frescas, em aparelho clevenger, e para determinar os principais constituintes foi realizada a cromatografia gasosa acoplada à espectrometria de massas. Foram realizados os testes de inibição da eclosão de ovos (TEO) e da inibição do desenvolvimento larval (TIDL). As concentrações utilizadas dos OEs em ambos os testes variaram de 75 até 0.2 mg/mL, sempre acompanhados de controle positivo com vermífugo comercial e controle negativo com água destilada. Os resultados indicaram que, quanto à constituição química, os componentes majoritários foram geraniale (*A. triphylla*), citronellal (*C. citriodora*), linalool (*L. alba*) e α-humuleno (*P. gaudichaudianum*). Para a eclodibilidade de ovos, os óleos de *A. triphylla* e *L. alba* apresentaram-se efetivos em todas as concentrações testadas, enquanto o óleo de *P. gaudichaudianum* apresentou-se eficaz até a concentração de 15 mg/mL e o óleo de *C. citriodora* até 10 mg/mL. Quanto ao desenvolvimento larval, os óleos essenciais pesquisados apresentaram-se totalmente efetivos nas concentrações testadas. Baseado nos resultados apresentados, conclui-se que os óleos essenciais avaliados têm potencial para utilização como anti-helmínticos naturais para tratamento de endoparasitoses em ovinos.

Palavras-chave: Anti-helmínticos; Ovinocultura; Verminoses

1 INTRODUCTION

Sheep production has grown in Brazil in order to guarantee the population a sorce of incoming, based mainly on the sale of meat. However, Gastrointestinal nematodes (GINs) are still the most prevalent parasites that cause disease in grazing ruminants worldwide, particularly in sheep (Girão *et al*., 1992; Coop; Kyriazakis, 2001, Osório *et al*., 2020, Beleckė *et al*., 2021).

For these reasons, these infections are responsible for huge economic and productive losses around the world (Abbas et al., 2020) and, in most cases, in the form of a subclinically, impaired weight gain as well as an impaired yield of meat and milk, but can also seriously endanger animal health and welfare by causing conditions such as diarrhoea, digestive problems, anorexia, anaemia, protein loss as well as reduced immunity, which can lead to death (Beleckė *et al*., 2021; Bosco *et al*., 2020, Abbas *et al*., 2020).

The treatment of these worms is usually carry out using drugs called anthelmintcs, but their excessive and indiscriminate use resulted in the selection and propagation of resistant parasites (Kaplan; Vidyashankar, 2012). According to Fortes; Molento (2013), the selection and growth of populations resistant to the main groups of anthelmintcs, avermectins, imidothiazoles and benzimidazoles (BZs), constitute a serious obstacle to the breeding of ruminants. Some studies have shown that Chemical dependence can be reduced through the integrated control of parasites, well as alternative treatments, for example the use of nematophagous fungi (Larsen, 1999), copper supplementation (Gonçalves; Echevarria, 2004), phytotherapic and the selection of animals genetically resistant to gastrointestinal parasitism (Minho *et al*., 2005). Among these alternatives, the selection of genetically resistant animals and the identification of plant bioactive compounds with anthelmintics action considered the most promising.

The use of vegetables with the aim of taking advantage of their wide range of possibilities has occurred since the beginning of humanity and may be sources of molecules capable of improving health conditions (Osório *et al*., 2021a). In this sense, the herbal medicine emerges as an effective alternative in this regard, becoming an option for the control of gastrointestinal parasitism in sheep and as a possible means of overcoming the forms of resistance to chemicals avaible for this purpose. Some medicinal plants produce secondary metabolites of great medicinal and economic importance, playing an important in health, industry and agriculture (Prochnow *et al*., 2017), standing out the essential oil, which are complex compounds that can contain different concentrations, often the dominant components determine the biological action of the oil (Felix, 2017).

In this way, the species *Aloysia triphylla* (L'Herit) and *Lippia alba* (Mill) are important because are excellent producers of essential oils, which makes them attractive to the industry (Hohlenwerger *et al.*, 2017; Zeppenfeld *et al.*, 2017). Both species have several and important biological actions well us *Piper gaudichaudianum* (Kunt), with an anti-inflammatory, fungicide, liver disease and analgesic (Moreira *et al.*, 2001; Di Stasi; Hiruma-Lima, 2002; Lago *et al.,* 2004; Morais *et al.,* 2007). Just like *Corymbia citriodora* (Hook), that essential oil an important source of biological active compounds, especially for disinfecting environments (Silva *et al*, 2017).

However, in veterinary medicine, the study of drugs produced from natural resources for disease control still enough explored. In addition, in recent years, there has been growing demand for less toxic products and food free of chemical residues, which encourages the search for bioactive molecules that can be used to control diseases and have less impact on the environment, enabling the offer of healthier products (Santos, 2013; Pietrobelli, 2019).

The in vitro tests allow an analysis of the existence desired properties in plants and constitute a preliminary step to the characterization of new active compounds presents, enabling the creation of new alternatives for the control of diseases, such as parasitic diseases (Costa *et al*., 2002). For the possible new anthelmintic considered effective, during egg hatchability tests, it must be able to prevent the larvae from hatching and being release into the environment. Therefore, the ideal is to have a product that prevents the blastula phase from proceeding. According to the classification of efficacy index, proposed by the World Association for the Advancement of Veterinary Parasitology (WAAVP) for adults parasites, a product is considered effective when to promote above 90% of anthelmintic action, moderately effective when acting between 80 to 90%, little effective when the action is between 60 to 80% and not effective at levels below 60% (POWERS *et al*., 1995).

Thus, this study aimed to evaluate the anthelmintic activity of essential oil extracted from plants species to obtain an effective bioproduct in the control of gastrointestinal parasites in sheep, able to circumvent the problem of resistance to conventional drugs and be exempt of additional chemicals.

2 MATERIALS AND METHODS

2.1 Plant material

The vegetables used for extracting essential oils belong to the germplasm bank of *Aloysia triphylla (A. citriodora)*, *Corymbia citriodora*, *Lippia alba* e *Piper gaudichaudianum (Piper aduncum L.)*, of the Aromatic Extractives Laboratory, belonging of Agronomic and Environmental Sciences Department, at the Federal University of Santa Maria, Frederico Westphalen Campus, RS.

2.2 Obtaining essential oils

The essential oils were extracted from the green mass of the leaves by the hydro distillation method in Clevenger, also in the Aromatic Extractives Laboratory. The oils were stored in an amber bottle at -4°C in a freezer. Chemical analysis was obtained by gas chromatography coupled with mass spectrometry was also performed.

2.3 Analysis of OEs constituents

The chemical composition of the essentials oils was determined using a gas chromatography-mass spectrometry system (6890N GC-FID, equipped with a DB-5 capillary column measuring 30 mm × 0.25 mm, and mesh thickness of 00:25 mm) connected to an FID Detector (Agilent Technologies, Santa Clara, California, United States). The detector and injector temperatures were adjusted to 280 °C using helium gas at a flow rate of 1.3 mL min^{-1}. The temperature programmed to go from 50 °C to 300 °C, at a rate of 5 °C min^{-1}. For chromatography experiments, 1 µL of each essential oil extract were used.

2.4 Egg recovery for biological tests

The eggs were recovered according to the methodology described by Coles *et al*., (1992), modified by Biziminyera *et al*., (2006). Feces were homogenized in water and filtered through a set of sieves (250, 75, 43, 25 µm), and the eggs were separated by means of centrifugation (in 3000 rpm, for 5 minutes) with a sodium chloride saturated solution. After this step, the eggs washed and used in the study. The L3 were obtained by stool test (fecal culture), following the technique of Roberts and O'Sullivan (1950) modified by Ueno and Gonçalves (1994).

2.5 Egg eclodibility assay (EEA)

This in vitro test was based on the method described by Coles *et al*., (1992) and standardized by Von Samson-Himmelstjerna *et al*., (2009), and assesses the ovicidal activity of the tested solutions. The concentrations ranged from 75 at 0.2%. mg.mL^{-1}. In addition, three controls were did: negative control, with distilled water; positive control, with a solution of 0.63 mg.mL of cydectin; and a control with TW. A suspension of 500 µL containing approximately 100 eggs was distributed in cell culture plates of 24 wells; afterwards, 500 µL of the test solutions were added per well. Subsequently, the plates were incubating in an oven for 24 hours at 27ºC. After the incubation period, drops of low Lugol (1%) were add to each well, and the eggs and larvae of first stage (L1) were quantify using an inverted optical microscope reading using objective 10x. The test was validated when the negative control and the control of TW had a greater than 90% hatching. To calculate the percentage of inhibition of larval hatching (Coles *et al*., 2006), the formula (%) inhibition = (A - B) / (A) × 100 was used, where: A = % of larvae hatched in the negative control and B = % of hatching in test solutions. The tests were performer in quadruplicate.

2.6 Larval development test (DLT)

For the larval development test based on the method described by Chagas *et al.* (2011). The 24 well plates received an aliquot containing approximately 100 eggs and completed it with enough distilled water at 400 µL. the plates were incubated in an oven for 48 hours at 27ºC. Afterwards, the larvae hatching was checked and 160 µL of the nutrient medium (*Escherichia coli*, amphotericin B and the fermentative extract) and 440 µL of solution-test in concentrations ranged from 75 at 0.2%. mg.mL. In addition, three controls were done: negative control, with distilled water; positive control, with a solution of 0.63 mg.mL of cydectin; and a control with TW, all with four replicates. Third stage larva was performer after culture larva collection, which was add Lugol and was visualize in inverted microscope. The inhibition percentage was given by the following formula: % larval development inhibition = (number of larvae in the control - number of larvae in treatment) / number of larvae in control x 100.

2.7 Statistical analysis

Analysis of Variance (ANOVA), followed by multiple comparison Tukey's test, was applied at a 5% probability level using the GraphPad Prism version 7.0 software. The dates were determined from the concentration-response curve, with a 95% confidence interval by means of the GraphPad Prism software version 5.0 for Windows.

3 RESULTS AND DISCUSSION

Currently, in vitro egg hatchability and larval development tests, in addition to verifying the action of synthetic anthelmintcs, have been use to screen aromatic plants, with the advantages of ease of execution, low cost and speed. These assays serve as an initial indication of the activity to investigate and allow the selection of the most promising compounds, reducing costs, avoiding waste of time and the indiscriminate use of experimental animals (Camurça-Vasconcelos *et al*., 2007). Thus, to analyze the effectiveness of the essential oils obtained, the in vitro egg-hatching test carried out, the results of which are show below, in Table 1.

As recommended by Hammond *et al.* (1997), in vitro tests allow the analysis of plants when in direct contact with the egg or larval stages of the parasite to evaluate their effect on the hatching of eggs, and these results are important to make these products, obtained from plants, as herbal medicines. Considering that for an anthelmintic treatment to considered efficient, in egg hatching tests, it must alter normal development, preventing the eggs from hatching and the release of larvae that become infective, being able to prevent the continuation of the phase of blastomeration. In this sense, the higher the percentage of eggs with arrested development retained in this phase, the greater the effectiveness of the treatment.

Table 1– Efficacy values of essential oils studied in vitro hatchability test of helminths eggs

Concentrations/ Essential oils (mg/mL)	Aloysia triphylla	Corymbia citriodora	Lippia alba	Piper gaudichaudianum	Commercial drug
75.0	100^{ns}	97.25^{ns}	100^{ns}	100^{ns}	100
50.0	100^{ns}	93.75^{ns}	100^{ns}	100^{ns}	100
25.0	100^{ns}	93^{ns}	100^{ns}	93^{ns}	100
20.0	100^{ns}	92.62^{ns}	100^{ns}	90.25^{ns}	100
15.0	100^{ns}	90.47^{ns}	100^{ns}	87*	100
10.0	100^{ns}	85.3*	100^{ns}	84.9*	100
5.0	100^{ns}	84.6*	100^{ns}	81.6*	100
4.0	100^{ns}	83*	100^{ns}	81.3*	100
3.0	100^{ns}	81.5*	100^{ns}	81.15*	100
2.0	100^{ns}	81.27*	99.17^{ns}	71*	100
1.0	100^{ns}	79.9*	97.22^{ns}	67.5*	100
0.8	100^{ns}	78.4*	96^{ns}	70*	100
0.6	100^{ns}	75.6*	94.25^{ns}	64.5*	98
0.4	99^{ns}	74.25*	93.5^{ns}	63*	97.75
0.2	98.5^{ns}	71.7*	89.75^{ns}	58.25*	97.5
0.0	5.1	5	5.3	4.6	5.9

*The treatment mean differs significantly ($p<0.05$) compared to the commercial drug mean by Dunnett´s test.
^{ns}The treatment mena does not differ significantly from the commercial drug mean by Dunnett´s test

Source: Authors (2022)

Given the above and analyzing the results presented in Table 1, according to the proposed classification by WAAVP, described by Powers *et al*. (1982). The essential oils of *A. triphylla* and *L. alba* were effective on the hatching of eggs, since both presented rates of 100% in the inhibition of embryonic development, even at lower concentrations. Should be considered capable of interrupting the continuation of the natural evolution of eggs, still in the early stages of helminth development, as they are above 90% of effectiveness indicated as indicators of the efficiency of anti-parasitic products.

Also according to the WAAVP classification, the essential oil of *C. citriodora* at concentrations ranging from 75 to 15 mg/mL considered effective, whereas between concentrations 10 to 2 mg/mL it was moderately effective and between concentrations 2 to 15 mg/mL. 0.2 mg/mL was not effective. *P. gaudichaudianum* under the same classification, up to a concentration of 20 mg/mL was effective, at a concentration of

15 mg/mL moderately effective, at concentrations ranging from 2 to 0.4 mg/mL, little effective and at the lowest concentration tested, 0.2 mg /mL was not effective.

It is observed that such results for *A. triphylla* and *L. alba* are equivalent to those obtained for the commercial drug, equally capable of stopping embryonic development in the blastula stage, even at low concentrations, as recommended by Hammond *et al.* (1997). This was not observed in analyzes for the *Corymbia citriodora* and *Piper gaudichaudianum* oils, which had their effectiveness reduced as their concentrations decreased, allowing the larvae to hatch. Therefore, these were able to inhibit the development of eggs only when in higher concentrations.

Regarding larval activity, it is possible to observe that the four species tested were effective as larvicidal agents, in vitro, as shown in Table 2, since the larvae were inactive and some with the cuticular structure disintegrated. The commercial drug moxidectin also showed 100% efficacy against the larvae and the negative control validated the results, since the larvae without the addition of the chemical or essential oils, remained viable after six days of incubation in the greenhouse, considered acceptable, since that the negative control showed results below 10% larval mortality.

Table 2 – Efficacy values of essential oils *in vitro* development test of helminth larvae, at different concentrations

Concentrations/ Essential oils (mg/mL)	**Aloysia triphylla**	**Corymbia citriodora**	**Lippia alba**	**Piper gaudichaudianum**	**Commercial drug**
5.0	100	100	100	100	100
4.0	100	100	100	100	100
3.0	100	100	100	100	100
2.0	100	100	100	100	100
1.0	100	100	100	100	100
0.8	100	100	100	100	100
0.6	100	100	100	100	100
0.4	100	100	100	100	100
0.2	100	100	100	100	100
0.0	2.88	5.9	0.88	3.5	4.0

Source: Authors (2022)

As recommended by Amarante (2014), the reduction in the number of infective larvae is one of the main targets of parasite control, which was observed in the present study. Therefore, even though they are not very effective as ovicides, the essential oils of *C. citriodora* and *P. gaudichaudianum* showed promise as biocontrols.

Believed that the results obtained in the ovicidal tests were due to the fact that not all essential oils were able to penetrate them, significantly decreasing their action as the concentrations decreased, because according to Homero (1984), nematode eggs they have an inner lipoprotein layer, an intermediate chitinous layer and an outer vitelline layer, making penetration difficult, precisely to make them resistant to adverse conditions. However, they may be susceptible to the action of lytic enzymes that degrade the chitin of eggs, and chitinolytic activity is probably the most relevant for egg sheath injury, which may have been the means of action of the essential oils of *A. triphylla* and *L. alba*.

Batista *et al.* (1999) demonstrated an inhibitory action of 50% on the hatching of *Haemonchus contortus* eggs obtained from sheep treated with aqueous extracts of *Spigelia anthelmia* and *Momordica charantia*. This last species also was teste by Costa *et al.* (2006), who used the ethyl acetate fraction and obtained up to 100% inhibition of the hatching of eggs of the same parasite. Assis *et al.* (2003) reported 100% inhibition of egg hatching using the ethyl acetate fraction. In Piauí, Girão and Carvalho (1999) tested aqueous extracts of *Luffa operculata* (bucha-paulista), *Operculina* sp (purge potato), *Momordica charantia* (São Caetano melon) and *Croton* sp (canopy) and observed inhibition of ruminant nematode egg hatching. Pessoa *et al.* (2002) using the essential oil of *Chenopodium ambrosioides* and *Ocimum gratissimum* on *H. contortus* eggs from goat feces, obtained 99.8 and 100% inhibition of egg hatching, respectively. They also confirmed the anthelmintic activity of *Ocimun gratissimum* essential oil and its component, eugenol, on *H. contortus* eggs. Ketzis *et al.* (2002) found that *Corymbia ambrosioides* essential oil is effective in preventing the hatching of nematode eggs from goat feces. Camurça-Vasconcelos *et al.* (2007) when they evaluated the ovicidal activity of the essential oil of *Lippia sidoides*, they also obtained total efficacy at concentrations of 2%, 1% and 0.5%. Likewise, Castro *et al.* (2017) found similar results to this study in the inhibition of egg hatching, when they used the essential oil of *Ocimum basilicum*, which showed action on eggs at all concentrations evaluated (0.25 mg/mL to 32 mg/mL). Also Grando *et al.* (2016), when testing the essential oil of *Melaleuca alternifolia*, obtained 100% in the hatch inhibition test.

Although the plant species used by the authors above are not the same as those tested in the present work, the results obtained with the essential oils of the *Lippia alba* and *Aloysia triphylla* plants are similar. This indicates the need for research that addresses the full potential of plants and reaffirms the importance of phytotherapeutic research in the area of veterinary parasitology. It is noteworthy that the results obtained statistically, for the essential oils of *Aloysia tryphilla* and *Lippia alba*, did not differ significantly from the commercial drug used as a positive control, at the concentrations tested, demonstrating their potential when tested against the eggs of the parasites.

Thus, these essential oils proved to be effective to solve the problem object of this work, since they were able to prevent the hatching of larvae, and, even in the face of these, were able to stop the development of the free life stages of these. helminths, which could be significant for the epidemiological control of these parasites, which are so frequent in the gastrointestinal system of sheep and that cause serious losses to producers and whose resistance raises concerns about the health of the herd (Max, 2010; Osório *et al*., 2021b), demonstrating that the use of plants to control nematodes that affect the gastrointestinal system of these animals is a viable alternative.

Generally, essential oil is attribute to the action of its dominant component. However, this reality is sometimes no repeated. Studies show that the effects do not always occur through this route, but through a synergy of the components, producing a new activity (Wolffenbuttel, 2016). The performance of essential oils depends on their components, which vary according to the botanical family of the vegetable, its origin, portion used, and growth stage and even according to the time of year that it was extracted (Gobbo-Neto; Lopes, 2007; Prochnow *et al*., 2017). From the technique of gas chromatography coupled to mass spectrometry, it was possible to indicate the chemical composition of the OEs of this work, which are the dominant components mentioned in Table 3.

Table 3 – Dominant chemical constituents, in percentage (%), of essential oils obtained by gas chromatography coupled to mass spectometry

Aloysia triphylla	***Corymbia citriodora***	***Lippia alba***	***Piper gaudichaudianum***
Geraniale (26.81)	Citronellal (80.26)	Linalool (64.95)	α-humuleno (13.65)
Limoneno (20.22)	Citronelol (8.50)	(E)-carvona (3.63)	(E)-nerolidol (7.99)
Neral (19.88)	Isopulegol (3.67)	γ-gurjuneno (3.19)	γ-gurjuneno (7.35)

Source: Authorship (2022)

The chromatographic alternatives that in *Aloysia triphylla* indicate the dominant components were in (2%): geranial (20.22), and spathulenol (5.21). In other studies of the composition of the essential oil of *A. triphylla*, they identified geranial (29.54%), neral (27.01%), limonene (15.93%), geranyl acetate (4.0%) as the predominant constituents. and geraniol (3.96%) (Figueiredo *et al*., 2004). According to the authors, this species has a higher amount of monoterpenes and a small amount of sesquiterpenes. In another chromatographic analysis, the constituents present in the essential oil of *Aloysia triphylla* were: geranial (28.39 to 36.90%), neral (19.17 to 27.55%), limonene (11.20 to 16.15%), nerolidol (5.23 to 12.34%), plus E-β-ocimene, zingiberene, caryophyllene, β-pinene, sabinene, germacrene D, ar-curcumene, geranyl acetate, terpinen-4ol, α-terpineol, linalool, trans limonene, α-oxide-pinene, myrcene and cis-oxido-limonene in lower values. The mixture of isomers (geranial and neral) forms citral, the main constituent of the essential oil of *Aloysia triphylla* (Lorenzi; Matos, 2008). In line with the data pointed out by Paulus *et al.* (2013), the chemical components of *A. triphylla* oil vary according to plant spacing and harvest times.

In *Corymbia citriodora* the predominant constituent was citronellal (80.26), with a lower predominance of citronellol (8.5) and isopulegol (3.67). These results were expect, since the essential oil of this vegetable, when extracted from leaves, has citronellal as its main component, used in perfumery, cleaning, food and pharmaceutical industries, according to Oliveira *et al.* (2014). This also justifies its larvicidal action, as Chagas *et al*. (2002) attributed the citronellol component, present in this oil, to the acaricidal action found in citronella oil.

In *Lippia alba*, the element that stood out was linalool (64.95), followed in smaller amounts by (E)-carvone (3.63), γ-gurjunene (3.19), α-calacorene (2.48) and (E)-dihydrocarvone (2.13). In an analysis carried out by Bahl *et al*. in 2000, for *L. alba* plants grown in India, showed that the dominant constituent of the essential oil (65%) was also linalool, as the outstanding component of the essential oil of the leaves of *L. alba*, among the 38 components identified in the study. The authors point out that since linalool is the priority component, the leaves of the plant can considered as a good source of alcoholic monoterpene with aromatic value for perfumes and food flavors. Linalool is not always referred to as the predominant component of the essential oil of *L. alba* leaves, as Rao *et al.* (2000) identified 50 components corresponding to approximately 90% of the total oil of the species and indicated geranial (15.57%), followed by a mixture of myrthene and myrtenal (9.89%), neral (9.44%) as the dominant component. %), geraniol (7.36%), 2,6-octadien-1-ol, 3,7-dimethyl acetate (6.87%), 1-octene-3-ol (4.66%), 6- methyl-5-heptene-2-one (4.60%), caryophyllene oxide (4.52%), b-caryophyllene (3.09%), citronellol (2.63%), linalool (2.20%), 3-pipene2-ol (2.19%), beta myrcene (1.49%), farnesol (1.35%) and spathulenol. In 2001, Lorenzo *et al.* analyzing the composition of the essential oil of the aerial parts of *L. alba* indicated linalool as the dominant component (55%), secondary component in our findings. This may even explain the result obtained in the TEO when using *L. alba* oil, since the possible mechanism of action is associated with linalool and involves protein denaturation and damage to the cytoplasmic membrane, which may explain the effectiveness against helminth eggs present in sheep feces (Camargo; de Vasconcelos, 2015).

In relation to *Piper gaudichaudianum*, α-humulene (13.65%) stood out, followed in smaller amounts by (E)-nerolidol (7.99%), γ-gurjunene (7.35%), (E)-caryophyllene

(6.49%) and α-selinene (5.16%). In verification carried out in some municipalities of Rio Grande do Sul, in order to indicate the main constituents of the essential oil of *P. gaudichaudianum* extracted from leaves and inflorescences, it also pointed out α-humulene as an outstanding component, in line with the result obtained in our analysis, being, therefore, the predominant element in samples of this plant in the state of Rio Grande do Sul (Silva *et al*., 2006; Péres *et al*., 2009).

As for the cytotoxicity of the extracted essential oils, information was enough in the literature on studies related to this topic, because although they are species already widely used in folk medicine, including for use in humans, there is always a need to certify the cytotoxic potential of substances with indication of use in animals. In this search, a study found in which the essential oil of *A. triphylla* added to fish water as a sedative for transporting the animals, in which no injuries or mortality occurred, even under exposure of the animals for 6 hours to the oil (Daniel, 2014).

In experiment whose objective was to test *C. citriodora* oil as an antiparasitic against ticks, the results indicated that eucalyptus essential oil acts on the different stages of parasitic life of the bovine tick (Olivo *et al.,* 2013), and between the groups of untreated animals and those that were bathed with a solution containing the essential oil of *C. citriodora*. There was similarity both in the organoleptic properties of the milk and in the physiological variables of the animals, indicating that it did not harm the health of the tested animals, nor to the product supplied, in this case, the milk.

As for the essential oil of *L. alba*, its efficacy has been reported in the treatment of spasmodic visceral diseases (Blanco *et al*., 2013), providing an antigenotoxic effect (López *et al*., 2011); insecticidal action (Peixoto *et al*., 2015) and action against tumor cells (Gomide *et al*., 2013). This makes its use interesting as an anticancer and reliable against healthy cells. In data presented by Cunha *et al.* (2010), at the concentrations described in their analyzes as being effective to generate anesthetic effects in fish, and especially at the concentration indicated as optimal (300 μg/mL), it did not show damage to the DNA of fish or mice, increasing in this way, the possibilities of anesthetics of natural origin, effective, non-toxic. According to the data presented by Kampke (2018), *L. alba* oil showed a small genotoxic effect in fish and mammals. WHO and ANVISA recommend genotoxicity assays as a requirement for the release of agents such as drugs, therefore, the essential oil of *L. alba* is indicated as an excellent anesthetic candidate for routine use in fish farming. In addition, its antiulcerogenic activity indicated by Pascual *et al.* (2001), showed that the activity presented by the infusion of the leaves did not change the gastric pH or the total acidity in male Wistar rats.

In a study promoted by Tomasini (2018), the essential oil of *P. gaudichaudianum* showed not to be cytotoxic in the non-tumor H9C2 strain, in 96 hours. However, it showed antitumor activity in the MCF7 lineage treated for 96 h, without increasing the generation of superoxide in 16 hours in these cells, have been point out how a good candidate for adjuvant in chemotherapy with doxorrubicin. Therefore, according to this study, *Piper gaudichaudianum* essential oil proved to be a good source of sesquiterpenes, with selective antitumor effect, as it did not show cytotoxicity in the non-tumor cardiomyocyte lineage. In another study, it was demonstrated that the essential oil of *P. gaudichaudianum* and its dominant components showed cytotoxicity in MCF7 cells (human mammary adenocarcinoma) (Manini *et al*., 2015), showing promise as a chemotherapeutic, however, non-toxic when in contact with benign cells.

Considering the current problem of parasitic resistance and the insertion of new molecules with an anthelmintic pharmacological principle. As well the search for the example of products that have low or no deficiency to products of animal origin, as well as to the environment (Sutherland; Leathwick, 2011), finding four larvicidal essential oils, two of which are also ovicidal, meets the objective proposed for this study.

4 CONCLUSIONS

In agreement with the results obtained, we can conclude that regarding the chemical constitution, the dominant component found in the essential oil of *A. triphylla* was geraniale (26.81%), while in *C. citriodora* it was citronellal (80.26%), in *L. alba* it was

Linalool (64.95%) and in the essential oil of *P. gaudichaudianum* it was α-humulene (13.65%). As for the anthelmintic activity on eggs and larvae obtained from the feces of naturally infected sheep, the essential oils of *Aloysia triphylla* and *Lippia alba*, under the conditions in which they were tested, showed efficacy equivalent to the commercial drug, as well as the molecules originated from them. Can become great allies in the control of nematodes that cause so many problems in Brazilian and worldwide sheep farming. The possible use of these plants in the future as an option or complement to traditional methods already established, in addition to the health impact, would bring possible benefits to the population increasingly concerned about the origin of the animal protein consumed, supporting and leveraging research for the use of herbal medicines and natural products. In addition, the larvicidal activity presented by the essential oils of *Corymbia citriodora* and *Piper gaudichaudianum*, allows their use as environmental decontaminants, since they acted with great effectiveness against the larvae that infect the gastrointestinal tract of sheep. The results suggest the possible role of tested EOs as anthelmintic agents in sheep farms, although further in vivo tests are needed.

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