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Effectiveness of Asteraceae extracts on Trichostrongylidae eggs development in sheep

Eficácia de extratos da família Asteraceae no desenvolvimento dos ovos de Trichostrongilídeos de ovinos

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Abstract

Data on *in vitro* evaluation of extracts of three species of the Asteraceae family on the development of Trichostrongylidae eggs in sheep are presented. Egg hatchability was tested using herbal extracts prepared in a Soxhlet extractor, and using hydrolate prepared by means of hydrodistillation. The laboratory tests showed that the ethanol extract from flowers of the species *Aster lanceolatus* presented high activity against Trichostrongylidae eggs development in sheep, inhibiting larva formation by 91% within 48 hours, and maintaining similar rates after 72 hours.

Keywords: Asteraceae, parasites, anthelmintic, phytotherapeutics.

Resumo

Apresentam-se dados da avaliação *in vitro* de três espécies vegetais da família Asteraceae sobre o desenvolvimento dos ovos de Trichostrongilídeos de ovinos. Realizou-se o teste de eclodibilidade com extratos vegetais preparados por aparelho de Soxhlet e hidrolato preparado por hidrodestilação. Os testes laboratoriais evidenciaram que o extrato etanólico das flores da espécie *Aster lanceolatus* apresenta alta atividade sobre o desenvolvimento dos ovos de Trichostrongilídeos de ovinos, inibindo em 91% a formação da larva em 48 horas, mantendo-se índices próximos em 72 horas.

Palavras-chave: Asteraceae, parasitas, anti-helmíntico, fitoterápicos.

Introduction

Most sheep flocks are infected with Trichostrongylidae that has high tolerance to chemotherapy. This gives rise to yield losses, not only both because of the support treatments that the infected animals need and increased manpower needs, but also especially because of the consequences of the high mortality rate within flocks (VAN WYK; MALAN, 1988; ECHEVARRIA et al., 1996).

The problematic resistance presented by nematodes to anthelmintics is a matter of worldwide concern (WALLER, 1994). Selection of species that are resistant to several types of anthelmintics may be attributed to intensive use of these products, underdosing or handling problems (RAMOS et al., 2002). Species of the family Trichostrongylidae originating from sheep, which were resistant to levamisole, ivermectin, and albendazole, were found in the state of Santa Catarina, in Brazil (RAMOS et al., 2002), and some species resistant to oxfendazole, levamisole and ivermectin were found in the state of Ceará (MELO et al., 2003).

Another problem in using chemical products to control parasites is the impact on the environment. There is a need to search for other, efficient parasite control methods, since the harm caused by parasitosis makes animal production impracticable (MARI; FIEL, 1992). Thus, among the new alternatives for controlling helminthiasis in sheep, one option investigated has been to identify phytotherapeutics with anthelmintic action (JACKSON, 1993; WALLER et al., 1995; HERD, 1996; VIEIRA, 2004).

In these investigations, the criteria used to select plant species were their availability and/or easy obtainment, the environmental

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impact that their use would cause (CUNNINGHAM, 2001) and the existence of other studies relating to their biological activity.

The first species to be selected was *Aster lanceolatus* Willd., commonly known as the panicled aster, tall white aster or white field aster (LORENZI; SOUZA, 1995), which presents antibacterial activity against *Streptococcus pyogenes, Salmonella typhimurium* and *Staphylococcus aureus* (DIAS et al., 2005, 2006); fungicidal activity against *Fusarium oxysporum* and *Cylindrocladium spathulatum* (DIAS et al., 2006); and allelopathy against germination and growth of *Lactuca sativa* (DIAS et al., 2007).

The second species to be selected was *Chamomilla recutita* (L.) Rauschert, commonly known as camomile, since this is the most widely cultivated and most popular herb among small farmers in Brazil. It presents carminative, antispasmodic, and anti-inflammatory properties (RAMOS et al., 2004), as well as being recommended as a vermicide (SALOMON, 1992).

The third species chosen was *Vernonia scabra* Pers., commonly known as ironweed, because of its presence in the regular diet of ruminants in the interior of Brazil (COSTA et al., 2006).

Thus, in this paper, we present our investigation on phytotherapeutic alternatives for controlling parasitism in sheep by means of in vitro parasite egg hatchability.

Materials and Methods

1. Plant material

The selected species were gathered from different areas, according to their natural distribution and local availability. The material collected was identified in conformity with the standards of classical taxonomy, based on floral morphological characters and using several samples, whenever possible. The species *A. lanceolatus* was collected in the city of Holambra, São Paulo, in June 2003, and was registered under number 287063 at the Municipal Botanical Museum of Curitiba; *C. recutita* was collected in the city of Mandirituba, Paraná, in April 2004; and *V. scabra* was collected in the SESC Private Natural Heritage Reserve, in the Pantanal region of Mato Grosso, in June 2002, and was registered under number 46141 at the herbarium of the Department of Botany, Universidade Federal do Paraná.

2. Extract and oil collection

To prepare the extracts and oils, the species were subjected to dissection in a heater, at a temperature of 40 °C, for 24 to 48 hours. They were then kept in a dry place, away from direct sunlight and at temperatures ranging from 5 to 15 °C, until use, when they were ground up and weighed.

The *C. recutita* seeds (148.94 g) were subjected to extraction in a modified Soxhlet apparatus (CARVALHO, 2009) with hexane, obtaining an oil fraction of 19%. The aromatic oil consisted of the oil retained in the condensation water during the hydrodistillation process on aerial parts of the *C. recutita* plant, which presented an output of 0.25%. The oils were used in the hatchability test without dilution.

The flowers (560 g) and the stems and leaves (800 g) of *A. lanceolatus* were subjected to extraction in a modified Soxhlet apparatus (CARVALHO, 2009) with ethanol 96° GL. After extraction, the total volume of each of the products obtained was measured and then condensed in a rotary evaporator at reduced pressure, at a temperature of 50 °C and rotation speed of 90 rpm, with filtration under vacuum, to produce concentrates known as crude extracts (CE). These extracts (1 mL) were dried up and rendered soluble in 5% ethanol for use in the hatchability test.

The aerial parts of *V. scabra* (100 g) were subjected to aqueous extraction by means of heat maceration. After extraction, the resultant extract was filtered in cotton, to produce an aqueous extract that was used in the hatchability test.

The filtered extract products were subjected to dry residue determination, in which 1 mL of extract to be analyzed was placed in a weighed pan and then heated at 100 °C until constant weight was achieved (DIAS, 2005). The result was shown as the amount of solids in 1 mL. We obtained 0.0785, 0.0475 and 0.1022 $g.mL^{-1}$ of dry residue for the extracts of flowers, stems and leaves from *A. lanceolatus* and for the aerial parts of *V. scabra*, respectively.

3. Obtainment of helminth eggs

The method used was the modified method proposed by the World Association for the Advancement of Veterinary Parasitology (WAAVP) (COLES et al., 1992).

To obtain helminth eggs for testing with the plant samples, sheep feces containing eggs were used. These eggs had the typical morphological characteristics of eggs produced by the superfamily Strongyloidea, family Trichostrongylidae, in accordance with the modified method of Gordon and Whitlock (UENO; GUTIERRES, 1983).

The feces were collected directly from the rectal ampulla and were kept at room temperature. The material was homogenized using a hypersaturated saline solution, filtered in mesh sieves of 180 and 250 $mn.\mu m^{-1}$, and then centrifuged at 2000 rpm for two minutes. The supernatant material was transferred to another centrifuge recipient, and three consecutive washes with distilled water were performed. During the last wash, a small amount of distilled water was kept in the sediment, which was resuspended and transferred to test tubes in aliquots of 200 µL, containing approximately 1430 eggs.

4. Hatchability test

The method used for this stage was a modified version of the hatchability test to determine the efficiency of the anthelmintic, in accordance with Krychak-Furtado et al. (2005). The same amount to be tested was added to each test tube containing 200 mL of the egg solution. The tubes were incubated under saturated moisture in a heater at a temperature of 26 ± 1 °C, for 72 hours. The time elapsed from collecting the feces to beginning the incubation of the eggs under the action of the plant extracts was two hours, at the most.

The influence of the samples on egg development was evaluated after incubation periods of 48 hours and 72 hours, by transferring

Table 1. Average percentage inhibition of Trichostrongylidae eggs development in sheep and formation of larvae over 48 and 72 hours.

Sample	48 hours			72 hours		
	Egg*	Egg**	Larvae	Egg*	Egg**	Larvae
Water	0.500[a]	0.050[a]	99.440[d]	0.000[a]	0.000[a]	100.000[d]
Alcohol	0.135[a]	1.550[a]	98.305[d]	0.100[a]	0.070[a]	99.825[d]
Albendazole	91.950[c]	8.045[a]	0.000[a]	74.645[c]	25.350[ab]	0.000[a]
A. lanceolatus[sl]	1.670[a]	87.135[d]	7.695[ab]	2.795[ab]	58.795[b]	38.410[bc]
A. lanceolatus flowers	91.850[c]	6.450[a]	1.695[a1]	80.305[c]	11.370[ab]	8.310[ab]
C. recutita	5.140[a]	48.425[b]	46.205[c]	2.550[ab]	22.445[ab]	75.005[cd]
Aromatic essence	5.840[a]	67.320[c]	26.830[bc]	1.935[a]	10.950[ab]	87.115[d]
V. scabra	63.700[b]	31.080[b]	5.210[ab]	60.645[bc]	26.135[ab]	13.220[ab]

[sl] = stems and leaves, * = blastomeres, ** = larva eggs. Averages with the same letters in the same column were not different from each other in the Tukey test ($p < 0.05$).

the samples from the test tubes to Petri dishes and making readings under an optical microscopic at a magnification of 100X. All eggs present in the samples were evaluated and classified according to the development stage at which they were found, i.e. blastomeres, larvae in eggs and larvae outside of eggs, and whether these larvae were mobile or not.

The procedure was performed in duplicate and repeated with distilled water, 70% ethanol diluted to 5% and albendazole sulfoxide, at a density of 136 $mg.mL^{-1}$, as the control for the tests. The results were expressed as percentages of inhibition and were subjected to statistical analysis using the SISVAR software (FERREIRA, 2000). Statistical differences were determined by means of the Tukey test and the statistical significance level was set at $p < 0.05$.

Results and Discussion

For an anthelmintic treatment to be considered efficient in a hatchability test, it needs to modify the normal development of the eggs, through stopping them from hatching or inhibiting the emergence of first stage larvae. Thus, the greater the percentage of blastomeres observed over the period evaluated, the greater the efficiency of the treatment will be.

With regard to the duration of the observations, all previous investigations were done over only a 48-hour period, and the evolution was halted through addition of Lugol's iodine at the time of making the readings. In the present experiment, the observations were continued until reaching 72 hours, without interruption of the larval evolution. For this reason, in the present study, it was sought to identify extracts that, although effective over a 48-hour period, would also enable evolution to occur over the next 24 hours, i.e. such that these extracts would not inhibit but would only delay egg development. This study would also make it possible to observe any occurrence of the opposite effect, i.e. identifying extracts that would allow larval development over a 48-hour period, but would impede hatching over a 72-hour period. Thus, as stated by Batista et al. (1999), delayed hatching and the action of the extract on the larvae might result in making all eggs laid by the parasite nonviable.

Table 1 displays the results obtained. It can be seen from this that the essential oil of *C. recutita* (hydrolate) did not present satisfactory results, given that it allowed approximately 27% of the eggs to hatch within 48 hours. At the 72-hour reading, 87% of the larvae had hatched. Treatment of Trichostrongylidae eggs from sheep with an aqueous extract from *V. scabra* showed that this plant was capable of inhibiting egg development by around 61%, as observed after 48 and 72 hours. This result is greatly superior to the results found by Alawa et al. (2003), thus suggesting that there is a need for more detailed study on this genus, since two of its species appear to be promising for anthelmintic treatment. However, among the three species, ovicide properties similar to those of albendazole were observed over 72 hours.

Conflicting results for the same experiment were found by Costa et al. (2002) when working with ethanol extracts and *Mangifera indica* hexane extract: the first presented excellent ovicide activity, while the second presented none. In the same way, extracts obtained from the same species, but from different parts of the plant, also demonstrated very different effects. Our tests on *A. lanceolatus* stems and leaves (Table 1) showed that although the samples did not stop the formation of larvae, they inhibited hatching by 87%. On the other hand, the extract obtained from flowers inhibited the formation of larvae by 91% over 48 hours, and similar manifestations were maintained over 72 hours, with results that were similar to those from albendazole.

Studies on this species have revealed that sitosterol is present in the flowers, stems and leaves, while spinasterol and kaempferol galactoside rhamnoside are present in its flowers (DIAS, 2005). The analysis on the essential oils produced from the flowers revealed thirteen components: myrtenol, muurolene, naphthalene, bisabolene, lanona, spathulenol, caryophyllene oxide, cyclohexene carboxaldehyde, cedrenol, neoclovene, azulene, benzocyclobutene and hexahydrofarnesyl acetone. Among these, the highest concentration is presented by caryophyllene oxide (DIAS et al., 2009). Thus, further studies on the extracts of *A. lanceolatus* should be conducted, with the purpose of determining their toxicity and safety for use in sheep flocks.

Conclusion

In this experiment, the activity levels of gross plant extracts were investigated with the objective of classifying the anthelmintic potential of the selected plants against Trichostrongylidae eggs development in sheep. Out of the three species tested, only the gross ethanol extract obtained from *A. lanceolatus* flowers presented

efficient action on Trichostrongylidae eggs development in sheep. This study encourages further investigations on this species, as a means for parasite control in animals, or as a species to be cultivated commercially in order to support the herbal drug industry.

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