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Artigo Cientifico

Medicina Veterinária

Use of herbal medicines in control of gastrointestinal nematodes of small ruminants: efficacies and prospects

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ABSTRACT: It is mainly due to the resistance to conventional anthelmintics representing a barrier, being a constant challenge to search for new bases in the control of these parasites. Thus the use of phytotherapics becomes viable alternative in the fight against gastrointestinal nematodes, beyond promoting slow development of the resistance, it is biodegradable and does not cause environmental contamination. Thereby, the review aims to address the main results obtained in research on herbal drugs effective in the control of gastrointestinal nematodes of small ruminants.

Key words: anthelmintics, medicinal plants, gastrointestinal nematodes, small ruminants

Uso de fitoterápicos no controle de nematódeos gastrintestinais de pequenos ruminantes:

eficácias e perspectivas

RESUMO: O desenvolvimento de resistência a anti-helmínticos representa um dos principais entraves para o controle das endoparasitoses em pequenos ruminantes, sendo justificada a busca de novas alternativas no controle de helmintos. Dessa forma, o uso de fitoterápicos torna-se alternativa viável no combate aos nematódeos gastrintestinais por

promover um desenvolvimento lento da resistência, ser biodegradável e não causar contaminação ambiental. Desse modo, esta revisão visa abordar os principais resultados obtidos em pesquisas com fitoterápicos no controle de nematódeos gastrintestinais de pequenos ruminantes.

Palavras-chave: anti-helmínticos; plantas medicinais; nematódeos gastrintestinais; pequenos ruminantes.

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Introduction

The caprine and ovine culture is a socioeconomic activity very explored in Brazil, mainly in the Northeast region, representing an important source of money income for the population, however the gastrointestinal nematodes has been a public health concern to the productive chain, being one of the major causes of subclinical diseases, and production and economical loss (MOLENTO et al., 2011; VERÍSSIMO et al., 2012). This problem is more evident on the developing countries, where nutricional sources are frequently insuficient to the small ruminants and, as a result, there is a imunity decrease of the animal, leading to low productivity and high mortality due to parasitism (KNOX et al., 2006).

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The control of the endoparasites is essencial to the succes within the production of small ruminants. Recently, this control is done. mainly with synthetical antihelminthics that aims to reduce the animal level of infection. however, the high cost, the inappropriated usage, and the development of resistent populations, associated with the risk of contamination of animal products and the enviroment due to residues of these compounds has been stimulating the search for control alternatives. (TORRES-ACOSTA; HOSTE, 2008; VIEIRA, 2008; ATHANASIADOU et al., 2008;).

Among the alternative ways, there is the phytotherapy, that uses bioactive plants rich in compounds that resembles antihelminthics (MAX et al., 2009) and because it has a important role when mixed with another methods, promoving a sustentable control of the nematode infections (CAMURÇA-VASCONCELOS et al., 2008). The conventional paradigm of the parasites through the usage of a chemical basis must be replaced or associated with the search for alternative aproachs that offer a perspective which reduces the chemoprophylaxis and contributes to keep the antihelminthics efficiency of the recent drugs. (CEZAR et al., 2008; VIEIRA, 2008; FRED-JAIYESIMI et al., 2011). Within this scenario, it is necessary to develop studies that aims complementary alternatives instead of the tradicional methods of control (ADEMOLA; ELOFF, 2010).

Thus, this work is adressed to approach the main results obtained in ressearchs with phytotherapics that are proved to be effective in the control of small ruminant nematodes.

Development

Efficacy of phytotherapics in the control of small ruminant helminths

With the goal of contributing to the alternative control of nematodes in small ruminants, several researchs has speacially focused on the etnoveterinary medicine, by using plants of the popular medicine, evaluating its priniciples, efficacy and safety level (MONTEIRO et al., 2011).

Bioactive components of plants may act on different life stages of nematodes, acting since the egg hatching until the parasite fecundity (HOSTE et al., 2006), motility, development and larval unsheathing, resulting with decrease of small ruminant infection, proven by the reduction of eggs per gram of faeces (EPG) and/or decrease of the graze contamination (MONTEIRO et al., 2011; MUPEYO et al., 2011; MARTÍNEZ-ORTÍZ-DE-**MONTELLANO** 2010: et al.. HERNÁNDEZ-VILLEGAS et al., 2012).

Two approaches have been used to evaluate the antihelminthic effect of the phytotherapics: preparation of products (extracts, essential oils or isolated substances) to *in vitro* and *in vivo* evaluation and the administration of plants *in natura* to the animals with experimental and natural infeccion (ATHANASIADOU et al., 2007). Thus, studies have been conducted to validate the efficacy of medicinal plants, and the *in vitro* tests are the best to the initial evaluation and triage of products to verify the antihelminthic activity of new vegetal compounds (ASASE et al., 2005).

According to the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.)reccomendations the phytotherapics can be classified by its effects of action, such as highly effective with 98% of efficiency, effective (90-98%), moderately effective (80-89%), or poorly active with less then 80% (WOOD et al., 1995). The differences on the products efficacy is due to the chemical compositions of the phytotherapic (AL-ROFAAI et al., 2012). Table Thus. the 1 show some phytotherapics that are described as owner of antihelminthic action, as well as the realized tests, and its respective efficacy.

HERBAL	AVALUATION	TEST*	ANIMAL	EFFECT (%)	MAIN CONSTITUENTS	REFERENCE
Cocos nucifera	IT	EHT LDT	SH	100 99,77	Taninos	OLIVEIRA et al. (2009)
Plectranthus punctatus Maesa lanceolata	IT	EHT and LDT	SH	100	Saponinas Benzofenol	TADESSE et al. (2009)
Eucalyptus globulus	IT	EHT LDT	SH	99,3 98,7	Monoterpeno 1,8-cineol	MACEDO et al. (2009)
Musa spp.	IT	LDT	SH	96,9	Taninos	OLIVEIRA et al. (2010)
Eucalyptus	IV		SH	76,5		MACEDO et al. (2010)
staigeriana	·	EPG	SH	60,7	Limoneno	
Emulsão de óleo de laranja	IV	EPG	SH	97,4	Terpenos	MESQUITA et al. 2013) SQUIRES et al. (2010)
Eucalyptus citriodora	IT	EHT LDT EPG	GT	98,8 99,7 66,2	beta-citronellal	MACEDO et al. (2011)
Senna occidentalis	IT			99,3	-	
Leonotis ocymifolia				100	-	
Leucas martinicensis		LDT	SH	99	-	EGUALE et al. (2011)
Albizia schimperiana				85	-	
Myracrodruon urundeuva	IT	EHT	SH	97,73	Taninos	OLIVEIRA et al. (2011a)
		LDT		100		(2011a)
Anadenanthera colubrine Leucaena leucocephala Mimosa tenuiflora	IT	LDT	SH	100	Taninos	OLIVEIRA et al. (2011b)
Mentha piperita	IT	EHT			Menthol	
Cymbopogon martini	11	LDT	SH	99	Geraniol	KATIKI et al. (2011)
Cymbopogon schoenanthus		LDT			Geraniol	
Alpinia zerumbet	IT			97,5		
Tagetes minuta		EHT	SH	96,8	Taninos	MACEDO et al. (2012)
Mentha villosa				97,6		
Artemisia lancea	IT	EHT	SH	99	1,8-cineole (34.56%)	ZHU et al. (2013)
		LDT		93,6		
		TML		77		
Annona muricata	IT	EHT LMT	SH	84,91 89,08	Compostos Felólicos	FERREIRA et al. (2013)

Tabela 1. Herbal medicines proven effective *in vitro* and *in vivo* tests used to control gastrointestinal nematodes in small ruminants.

* Egg hatching test (EHT) and larval development test (LDT); EPG: eggs per gram of faeces; LMT: larval motility test; IT: *in vitro*, IV: *in vivo*, SH: Sheep, GT: goat.

In Brazil, studies shows the existence of different part of the vegetables that can be used as alternative on the antihelminthic control, such as aqueous extracts of leafs and pseudostem, essential oils and decoctions (OLIVEIRA et al., 2010; MACEDO et al. 2012; RIBEIRO et al., 2013).

Recent studies quoted the use of plants rich in secondary metabolites, several of those with high quantity of polyphenols, in special the tannins (HOSTE et al., 2012). Diverse biological actions are assigned to these substances that acts on the different parasite stages and the free live (MUPEYO et al., 2011).

Considering the use of tanniferous plants a promising alternative on the control of small ruminant nematodes, OLIVEIRA et al. (2011a) described the leaf extract and stem efficacy from *Myracrodruon urundeuva* on *H. contortus*, evaluating the ovicidal effect and the action of the extracts on the unsheathing of the third stage larvae (L3). The extracts

demonstrated dose dependent ovicidal effect, however the leaf extract has been more effective inhibiting 97.73% of the egg hatching on the concentration of 1,25 $mg.mL^{-1}$, while the stem inhibited 83.56% of the eclosion on the concentration of 5 $mg.mL^{-1}$. The action of tannin involved on antihelminthic activity the was also evaluated by the of use polyvinylpolypyrrolidone, responsiblefor the inhibition of tannins, confirming the role of these secondary metabolites on the exsheathment of L3. A similar study was developed by OLIVEIRA et al. (2011b), aiming evaluate the role of tannins found of tanniferous plants on extract Anadenanthera colubrina. Leucaena leucocephala e Mimosa tenuiflora on the process of L3 from *H. contortus*.

Promising results were also described after incubation of nematodes with vegetable extracts rich in tannins, affecting the egg hatching, development and larvae motility of *H. contortus* (BARRAU et al., 2005; BRUNET et al., 2007; AKKARI et al., 2008; MOLAN et al., 2010), as well as the reduction of egg removal on faeces (LANGE et al., 2006; HECKENDORN et al., 2007; MAX et al., 2009; JOSHI et al., 2011). There are some reports of reduction on parasite load of caprine and ovine nematodes fed with tanniferous plants (MINHO et al., 2008; MINHO et al., 2010).

MACEDO et al. (2012) evaluated the effect of decoctions rich in tannins derived from Lantana camara (Lc), Alpinia zerumbet (Az), Tagetes minuta (Tm) and Mentha villosa (Mv) on the inhibition of egg hatch from *H. contortus*. In the concentration of 2,5 mg.ml⁻¹, Tm and Mv demonstred efficacy of 96,8 and 97,6%, respectively, and did not differed statistically of the positive control, thiabendazole. In the same study, the authors related that Lc presented no effect on the egg hatching of H. contortus. FERREIRA et al. (2013) evaluated the acquus extract from the leaf of Annona *muricata*, popularly known as soursop fruit and it demonstred ovicidal effect on *H. contortus*, with 84,91% of efficacy, while diluted on the concentration of 50%. The test of larval motility had an inhibition of 89,08% on 12% dose. When tested in adults, there was a complete inhibition of motility on the first 6-8 hours of observation.

OLIVEIRA et al. (2009) evaluates the extract of ethyl acetate obtained on the liquid of the Cocos nucifera shell in egg hatchability and larvae development of H. contortus, atributing its action to the presence of tannins that promoted the infeasibility from tested eggs and with inhibition of 99,77% in larvae development on the concentrations of 5 $mg.mL^{-1}$ and 80 mg.mL⁻¹, respectively, and statistical difference had no when compared negative controls, to thiabendazole $(0.025 \text{ mg.ml}^{-1})$ for egg hatching and ivermectin $(0.008 \text{ mg.ml}^{-1})$ for larvae development.

The use of essential oils represents other option of phytotherapic approach and

are between the vegetable substance classes that reported antiparasitic activity (ANTHONY et al., 2005). MACEDO et al. (2011) evaluated the in vitro and in vivo activity of the essential oil from *Eucalyptus citriodora*, in caprine naturally infected. Results showed reduction of 98.8% on egg hatch and 99.71% on larvae development in H. contortus, on 5,3 and 10.6 mg.mL^{-1} doses, $mg.mL^{-1}$ respectively. On fecal egg count reduction test (FERCT), the mean efficacy was 66.25% being inferior when compared to ivermectin that showed 79.16% of antihelminthic action in caprine on the 8th day after treatment.

Elevated efficacy was also described to the essential oil of *Eucalyptus globulus* presenting the maximun action of 99,3% on the egg hatching tests and 98,7% on larvae development of *H. contortus*, on the concentration of 21,75 mg.mL⁻¹ and 43,5 mg.mL⁻¹, respectively (MACEDO et al., 2009). These results leads to the potential antihelminthic action of the oil of *E. citriodora* and *E. globulu* on the control of ovine and caprine nematodes.

KATIKI et al. (2011) tested the essential oil activity from Mentha piperita, Cymbopogon martinii and Cymbopogon schoenanthus in vitro, performing Egg hatching test (EHT), larval development test (LDT), larval exsheathment test (LET) and larval feeding inhibition test (LFIT) in different dilutions. From all the tests the LD50 and LD99 were tested. The C. Schoenanthus essential oil demonstred better activity against ovine trichostrongylids with LD₅₀ and LD₉₉ of 0,04 and 0,27 mg.mL⁻¹ for EHT, 0,06 and 0,27 mg.mL⁻¹ for LET, 24,66 and 5,23 mg.mL⁻¹ for LDT, and finally 0,009 and 24.66 mg.mL⁻¹ for LFIT respectively. The confirmation of the capacity of larvae development in vitro, becomes important over the control of nematodes enviromental cicle, decreasing the pasture contamination and consequently modulating the risk of parasite infection (MAX, 2010).

In a study using Eucalyptus staigeriana oil on a dose of 500 mg.kg⁻¹ administering during three days in caprine infected by H. contortus, demonstred EPG reduction ranging from 61,4 to 76,57% on days 8 and 15 after treatment. On the same period the ivermectin efficacy ranged from 85,59 to 67,34% (MACEDO et al., 2010). However, the volatility and insolubility of the Eucalyptus spp. oil has limited its use on nematodes control (BATISH et al., 2008). Therefore, more recently, the preparation of chitosan pharmaceutical formulas based on the utilization of matrices for encapsulation of volatile compounds, it has been proposed to promote a higher protection of the drug and maximize the biological effect of essential oils (MESQUITA et al. 2013; RIBEIRO et al., 2013), moreover, it grants a higher solubility in. Thus. the nanotecnology consists of a adequated approach to implement the phytotherapics, once the possibility of optimizing the

efficacy of these products exists (IRACHE et al., 2011).

In the attempt to validade vegetable products, Squires et al. (2010) tested in gerbils experimentally infected with H. contortus a emulsion of orange oil, and verified on the eighth day after infection a parasite reduction of 62.6% and 87.8% on the concentrations of 600 and 1200 mg.kg ¹, respectively, in a single dose. A dose of 600 mg.kg⁻¹ was tested in ovine, reducing 97.4% of the egg counted on faeces, when administered in a single dose and 94,9% when administered by three days in a row. Though the authors described promising results for the tested product, these must be interpreted with caution due to high doses and the number of administrations to obtain the wanted antihelminthic effected.

ZHU et al. (2013) after used the essential oil from *Artemisia lancea* described satisfatory results to EHT, LDT and LFIT over *H. contortus*. On EHT, the efficacy was 99,4% when used the dose of 10 mg.mL⁻¹ and its major component, 1,8-

Cineole, evidenciated moderated ovicidal activity (74,8%) with LD_{50} of 4,64 mg.mL⁻¹. On LDT, the essential oil of *A. lancea* and 1,8-Cineole, inhibited 93,6 and 65,2% on the dose of 10 mg.mL⁻¹, with LD_{50} 1,66 and 5,07 mg.mL⁻¹, respectively. And on LFIT there was a inhibition of 79,6 and 60,3% respectively, being all results dose dependent.

TADESSE et al. (2009) evaluated the action of acqueous extracts and hydroalcoholic from Maesa lanceolata and Plectranthus punctatus over the egg hatching and larvae development of H. contortus. All the extracts tested demonstred efficacy above 98,9% on EHT, na dose de 0.5 mg.mL^{-1} . When evaluated at $mg.mL^{-1}$, happened the complete 1 inhibition of hatchability in all tested samples. The extracts were also able to inhibit the larvae development, with the best DL₅₀ registered to the hydro-alcoholic extracts of leafs from *M. lanceolata*.

Similar tests were realized by EGUALE et al. (2011), to evaluate *Senna*

occidentalis, Leonotis ocymifolia, Leucas martinicensis, Rumex abyssinicus, and schimperiana Albizia on the egg hatchability test and larvae demonstrating complete inhibition of egg hatching in concentrations below 1 mg.mL⁻¹ to the acqueous and hydro-alcoholic extract of L. martinicensis, L. ocymifolia and acqueous S. occidentalis extract of and Α. schimperiana. On LDT, the aqueous extract of L. ocymifolia, L. martinicensis, schimperiana and S. occidentalis *A*. presented efficacy of 100, 99, 85, and 99,3%, respectively. While the hydroalcoholic extract of A. Schimperiana inhibited 99,09% on the maximun concentration tested (50 mg.m L^{-1}), the extract of S. occidentalis (9%) and L. ocymifolia (37%) demonstred low inhibition on the same concentration.

In vitro tests with different extracts (acqueous, methanolic and dichloromethane) from parts (leaves, fruit and stem) of *Tabernaemontana citrifolia*, a plant commonly used as antihelminthic in small ruminants in Guadalupe, France, demonstred efficacy, depending on the parasite stage of *H. contortus*. The major effect found was on LDT, with reduction of 99.8% for extracts from the fruit, 83.8% from roots and 85% to the leaves. On EHT and on the larvae motility test, the results revealed efficacy of 22.7% and 56%, respectively to the extract from roots. To the larvae migraton test the higher efficacy was of 49.4% to the leaves extracts (MARIE-MAGDELEINE et al., 2010).

The alternative of utilization of plants can be a useful tool associated to other methods of smalls ruminant nematodes control, having its use justified even with a efficacy inferior to 95% in situations where synthetic antihelminthics are not recomended, as in the organic livestock, dairy production, or when the cost is not compensatory (CAMURÇA-VASCONCELOS et al., 2008). Thus, plants with moredated antihelminthic activity must be considered, because they integrated allow approach, can а

specifically designed to reach a sustainable control of parasites on the ruminant systems of production. (GITHIORI et al., 2006).

Final Considerations

The research for new phytotherapic on the field of Veterinary Parasitology is important to the control of small ruminant nematodes. The study of new substances to the verification of antihelminthic activity, becomes justifiable by the necessity of a sustainable control, through the use of biodegradable and self sustainable Therefore, material. studies with phytherapics can contribute to expand the knowledge about antihelminthic actions of plants, allowing a better understanding of the fundamental aspects of its biological activities, as well as its constituents, which may constitute in a useful tool on the control of small ruminant parasites.

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