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Artigo Científico

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 phytotherapies 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 nutritional sources are frequently insufficient 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).

The control of the endoparasites is essential to the success within the production of small ruminants. Recently, this control is done, mainly with synthetic anthelmintics that aim to reduce the animal level of infection, however, the high cost, the inappropriate usage, and the development of resistant populations, associated with the risk of contamination of animal products and the environment 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 phytotherapy, that uses bioactive plants rich in compounds that resemble anthelmintics (MAX et al., 2009) and because it has an important role when mixed with another method, promoting a sustainable 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 approaches that offer a perspective which reduces the chemoprophylaxis and contributes to keep the anthelmintic 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 aim complementary alternatives instead of the traditional methods of control (ADEMOLA; ELOFF, 2010).

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

Development

Efficacy of phytotherapies in the control of small ruminant helminths

With the goal of contributing to the alternative control of nematodes in small ruminants, several researches have specially

focused on the etnoveterinary medicine, by using plants of the popular medicine, evaluating its principles, 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 unshathing, 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 et al., 2010; HERNÁNDEZ-VILLEGAS et al., 2012).

Two approaches have been used to evaluate the antihelminthic effect of the phytotherapies: 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 infection (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.) recommendations the phytotherapies 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 than 80% (WOOD et al., 1995). The differences on the products efficacy is due to the chemical compositions of the phytotherapeutic (AL-ROFAAI et al., 2012). Thus, the Table 1 show some phytotherapies that are described as owner of antihelminthic action, as well as the realized tests, and its respective efficacy.

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

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

* 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 the antihelminthic activity was also evaluated by the use of polyvinylpolypyrrolidone, responsible for 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 on extract of tanniferous plants *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 demonstrated efficacy of 96,8 and 97,6%, respectively, and did not differ 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 aqueous extract from the leaf of *Annona muricata*, popularly known as soursop fruit

and it demonstrated 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*, attributing 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 had no statistical difference when compared to negative controls, thiabendazole (0.025 mg.ml⁻¹) for egg hatching and ivermectin (0.008 mg.ml⁻¹) for larvae development.

The use of essential oils represents another option of phytotherapeutic 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 mg.mL⁻¹ and 10,6 mg.mL⁻¹ doses, 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 maximum 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. globulus* on the control of ovine and caprine nematodes.

KATI KI 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 demonstrated 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 environmental cycle, 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*, demonstrated 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 nanotechnology consists of a adequate approach to implement the phytotherapies, once the possibility of optimizing the

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

In the attempt to validate 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 effect.

ZHU et al. (2013) after used the essential oil from *Artemisia lancea* described satisfactory 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, evidenced moderated ovicidal activity (74,8%) with LD₅₀ 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 LD₅₀ 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 aqueous extracts and hydro-alcoholic from *Maesa lanceolata* and *Plectranthus punctatus* over the egg hatching and larvae development of *H. contortus*. All the extracts tested demonstrated efficacy above 98,9% on EHT, na dose de 0,5 mg.mL⁻¹. When evaluated at 1 mg.mL⁻¹, happened the complete 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 *Albizia schimperiana* on the egg hatchability test and larvae demonstrating complete inhibition of egg hatching in concentrations below 1 mg.mL⁻¹ to the aqueous and hydro-alcoholic extract of *L. martinicensis*, *L. ocymifolia* and aqueous extract of *S. occidentalis* and *A. schimperiana*. On LDT, the aqueous extract of *L. ocymifolia*, *L. martinicensis*, *A. schimperiana* and *S. occidentalis* presented efficacy of 100, 99, 85, and 99,3%, respectively. While the hydro-alcoholic extract of *A. Schimperiana* inhibited 99,09% on the maximum concentration tested (50 mg.mL⁻¹), the extract of *S. occidentalis* (9%) and *L. ocymifolia* (37%) demonstrated low inhibition on the same concentration.

In vitro tests with different extracts (aqueous, methanolic and dichloromethane) from parts (leaves, fruit and stem) of *Tabernaemontana citrifolia*, a plant commonly used as antihelminthic in

small ruminants in Guadalupe, France, demonstrated 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 migration 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 small ruminant nematodes control, having its use justified even with a efficacy inferior to 95% in situations where synthetic antihelmintics are not recommended, as in the organic livestock, dairy production, or when the cost is not compensatory (CAMURÇA-VASCONCELOS et al., 2008). Thus, plants with moderate antihelmintic activity must be considered, because they can allow an integrated approach,

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

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