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Espécies de plantas do cerrado selecionadas por ovinos em pastejo com potencial na inibição do desenvolvimento de *Haemonchus contortus*

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Belo Horizonte

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Tese apresentada ao Programa de Pós-graduação em Parasitologia como parte dos requisitos do Título de Doutora em Ciências

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Resumo

Espécies vegetais naturalmente selecionadas por ovinos no Bioma Cerrado (W 43º50'33.56" e S 16º41'10.05") foram avaliadas *in vitro* e *in vivo* para o controle de *Haemonchus contortus*. Após um ano de observações, as famílias das espécies que mostraram maior riqueza foram Fabaceae, Rubiaceae, Malpighiaceae, Bignoniaceae, Myrtaceae e Annonaceae. Houve variações do índice de selectividade para nove espécies vegetais selecionadas por ovinos nas estações seca e chuvosa. A coprocultura foi realizada em cinco repetições com 11 tratamentos: ivermectina, água destilada e folhas desidratadas de nove espécies de plantas (333,3 mg g^{-1} cultura fecal). Após sete dias, as folhas desidratadas de *Piptadenia viridiflora* e *Ximenia americana* foram eficazes em 86,5 e 99,8 % respectivamente reduzindo significativamente o número de larvas infectantes presentes na cultura em comparação com o controle com água destilada estéril. A presença de taninos e flavonóides do extrato aquoso e etanólico foi indicado por HPLC (*High-performance liquid chromatography*). Os extratos de *P. viridiflora* e *X. americana* (0,075-2,4 mg mL^{-1}) apresentaram eficácia de 69.6-100% no teste de eclodibilidade. Na inibição do desenvolvimento larval o extrato aquoso de *P. viridiflora* (1,21-38,62 mg g^{-1}) foi eficaz entre 55,63-100%, para *X. americana* (1.42-22.70 mg g^{-1}) foi eficaz entre 65,63-100% e significativamente menor que o observado para o controle com água ($P<0,05$) para ambas as espécies. A eficácia antihelmíntica *in vivo* foi entre 32,9-47,2% para *P. viridiflora*, para *X. americana* não houve eficácia. Valores de hemácias e hematócrito ficaram dentro dos padrões fisiológicos normais para ovinos tratados com *P. viridiflora*, para albumina plasmática e proteína total, efeitos significativas entre os tratamentos, não foram encontrados. *P. viridiflora* mostra potencial promissor como tratamento alternativo de haemonchoses. As baixas concentrações de extratos das folhas dessa espécie mostram alta eficácia para eclosão e desenvolvimento larval. O extrato aquoso administrado a 283 mg (ms) kg^{-1} de peso corporal durante três dias consecutivos promove eficácia de 47,15% para a redução da contagem de ovos nas fezes e sem distúrbios clínicos e sem alterações no sangue.

Palavras-Chave: Savana brasileira. Plantas antihelmínticas. Composição fitoquímica. Toxicidade. Parâmetros sanguíneos. *Haemonchus contortus*.

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Plants of the cerrado selected by grazing sheep with potential for inhibition of larva development of *Haemonchus contortus*

Abstract

Plant species naturally selected by sheep grazing in the Cerrado region of Brazil were assessed for *in vitro* or *in vivo* activity against *Haemonchus contortus.* After one year of observations, the plant families showing greatest richness in the region were Fabaceae, Rubiaceae, Malpighiaceae, Bignoniaceae, Myrtaceae and Annonaceae. Variation in the selectivity index for nine commonly plant species selected by grazing sheep was observed with respect to the dry and rainy seasons in the Cerrado. Coproculture was conducted in five replicates of 11 treatments: ivermectin, distilled water, or dehydrated leaves of nine selected plant species administered at 333.3 mg g^{-1} fecal culture (FEC). After seven days, the dried powder of *Piptadenia viridiflora* and *Ximenia americana* leaves, were effective at 86.5 and 99.8%, respectively, reducing significantly reduced the number of infective larvae compared to the control with sterile distilled water. The presence of tannins and flavonoids for aqueous and ethanolic extracts was indicated by HPLC (*High-performance liquid chromatography*). The extracts of *P. viridiflora* or *X. americana* (0.075-2.4 mg mL^{-1}) was between 69.6-100% efficacy in the egg hatch inhibition (EHI). In larval development inhibition (LDI), the aqueous extract *P. viridiflora* (1.21-38.62 mg g^{-1}) anthelminthic efficacies were between 55,63-100%, *X. americana* (1.42-22.70 mg g^{-1}) efficacies were between 65,63-100% and showed mean of L3 and significantly lower than those observed for the control with water ($P<0.05$), for both species. The *in vivo* anthelminthic efficacies were between 32.9 - 47.2% for *P. viridiflora*, no efficacy for *X. americana*. Erythrocyte and hematocrit values were within the normal physiological patterns for lambs for *P. viridiflora*. For plasmatic albumin and total protein, significant effects between treatments were not found. *P. viridiflora* shows promising potential as alternative treatment of haemonchosis. The low concentrations of leaf extracts show high efficacy to EHI and LDI. Aqueous extract administered at 283 mg (dm) kg^{-1} bw during three consecutive days promotes efficacy 47,15% to FEC reduction and no clinical or blood disorders.

Key words: Brazilian savannah. Anthelminthic plants. Phytochemical composition. Toxicity. Blood parameters. *Haemonchus contortus*.

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1 Introdução

A ovinocultura pode constituir uma alternativa econômica e viável em regiões do Brasil, gerando emprego e renda familiar, bem como fixando o homem no campo. No norte e nordeste de Minas Gerais, pode-se observar a situação precária do manejo sanitário adotado nos criatórios (Sebrae, 2004). Há dificuldades de realização de exames laboratoriais, seja pela falta de técnicos ou pela falta de laboratórios regionais. Vieira (2003), ao pesquisar propriedades no norte de Minas Gerais, verificou que em 94,9% dessas propriedades nunca tinha sido feito nenhum exame parasitológico nos rebanhos.

O principal problema encontrado na ovinocultura, e que limita o aproveitamento econômico desses animais são, as parasitoses gastrintestinais. A alta prevalência e grande patogenicidade fazem de *Haemonchus contortus* uma das principais espécies de endoparasitas de ovinos no Brasil e no mundo. *H. contortus* é o mais patogênico parasita do abomaso e alimenta-se de sangue durante toda vida parasitária. Os ovinos com haemoncoses podem apresentar anemia e edema submandibular e casos de mortalidade em filhotes e fêmeas parturientes ocasionadas por esses parasitos são relativamente comuns (Bizimenyera et al., 2006).

O tratamento frequente dos rebanhos com antihelmínticos tem sido a única medida de controle da verminose adotada pela maioria dos criadores. A administração constante e em doses inadequadas favorece a seleção de populações de parasitas resistentes aos princípios ativos e contribui para a contaminação dos produtos de origem animal com resíduos das drogas utilizadas (Amarante *et al*., 1992).

Os primeiros estudos referentes à resistência dos helmintos estão relacionados com o grupo dos benzimidazóis, imidazotiazóis e posteriormente as avermectinas, que surgiram como uma alternativa de tratamento que tem sido considerada até hoje, como um princípio ativo potente, para o controle das parasitoses de animais domésticos (Gopal et al., 1999). Muitas propriedades no país já apresentam populações resistentes a todas as bases disponíveis e esse fenômeno tem inviabilizado a exploração dos ovinos nessas áreas, pois enfrentam problemas que promovem queda na produção, disponibilidade e qualidade dos alimentos, deficiência mineral, manejo inadequado e elevada incidência das helmintoses gastrintestinais, o que ocasiona grandes perdas econômicas (Araújo e Lima, 2005; Geraseev et al. 2011; Duarte et. al., 2012).

A fitoterapia no controle de verminose é uma alternativa que poderá reduzir o custo com a aquisição de antihelmínticos bem como prevenir o aparecimento de resistência antihelmíntica e a presença de resíduos nos produtos de origem animal. Muitas espécies vegetais são tradicionalmente conhecidas como possuidoras de atividade antihelmíntica, necessitando, entretanto, que suas eficácias sejam cientificamente comprovadas (Vieira, 2003).

Em consequência da intensa exploração econômica do Cerrado, boa parte da vegetação nativa foi derrubada e muitas espécies estão ameaçadas de extinção, as quais podem apresentar ampla utilização e manutenção da população local por seu valor alimentício, medicinal, ornamental, oleaginoso e tanífero. Entretanto, poucos estudos têm avaliado o efeito antihelmíntico dos compostos químicos das espécies vegetais do Cerrado para o controle das helmintoses.

Os taninos podem exercer ação antihelmíntica direta, ao interferir no ciclo natural dos helmintos, ou indireto, ao proteger a proteína ingerida da degradação ruminal (com incremento da disponibilidade protéica no trato gastrintestinal inferior), o que dificulta a determinação do seu real efeito antiparasitário (Ketzis *et al*., 2006).

A validação científica de novas alternativas de produtos antihelmínticos é uma etapa inicial e obrigatória para testes *in vitro* e *in vivo* com espécies vegetais, o que vai permitir uma análise da caracterização de novos compostos ativos, possibilitando novas perspectivas para o controle das endoparasitoses de ovinos ou de outros animais domésticos.

Portanto, o estudo do uso alternativo de extratos de espécies vegetais no controle de helmintos em pequenos ruminantes pode constituir uma estratégia promissora para a indústria biotecnológica nacional e consequentemente para os criadores.

2 Justificativa

Um dos principais problemas limitantes para a ovinocultura e caprinocultura são as parasitoses gastrintestinais. Todas as categorias de ovinos podem ser intensamente parasitadas por helmintos, reduzindo não somente o ganho de peso, mas também a produção de leite, lã e pele. A alta prevalência e grande patogenicidade fazem de *Haemonchus contortus* e *Trichostrongylus colubriformis* uma das principais espécies de endoparasitas de ovinos no Brasil e no mundo.

Haemonchus contortus parasita a mucosa do abomaso e alimenta-se de sangue durante toda vida parasitária, ocasiona anemia, edema submandibular e elevadas taxas de mortalidade em filhotes e em fêmeas parturientes. O tratamento frequente do rebanho ovino com antihelmínticos sintéticos tem sido uma das únicas medidas de controle dos nematódeos gastrintestinais adotada pelos criadores de ovinos.

A utilização de antihelmínticos, além de elevarem o custo de produção, compromete o ecossistema através da persistência de seus resíduos no ambiente e nos produtos de origem animal, que de forma extremamente efetiva induzem a seleção de cepas de parasitos resistentes.

Assim, ao observar a dieta selecionada por ovinos naturalmente em área de Cerrado, Costa (2010), avaliou pelo índice de seletividade as espécies vegetais mais consumidas por esses animais durante a estação seca e chuvosa: *Acosmium dasycarpum* (Vogel) Yakovlev (Fabaceae), *Baccharis tridentata* Vahl. (Asteraceae), *Casearia sylvestris* Sw. (Salicaceae), *Paullinia* sp., (Sapindaceae), *Ximenia americana* L. (Olacaceae), *Erythroxylum deciduum* A.St.-Hil. (Erythroxylaceae), *Heteropterys byrsonimifolia* A. Juss. (Malphigiaceae), *Lippia sidoides* Cham. (Verbenaceae), *Schinopsis brasiliensis* Engl. (Anacardiaceae), *Evolvulus* sp. (Convolvulaceae), *Senna spectabilis* (DC.) H.S.Irwin & Barneby (Fabaceae) e *Piptadenia viridiflora* (Kunth) Benth (Fabaceae).

Contudo, poucos estudos têm avaliado o efeito antihelmíntico dos metabólicos de espécies vegetais, desse modo, emerge a necessidade da utilização de produtos naturais, a base de espécies vegetais encontradas nas regiões onde ocorre o pastejo de ruminantes. Sendo assim, torna-se importante testar novas bases farmacológicas para o controle desses parasitos.

3 Objetivos

3.1 Objetivo geral

Avaliar o potencial antihelmíntico de espécies vegetais do Cerrado selecionadas naturalmente por ovinos no norte de Minas Gerais.

3.1 Objetivos específicos

- Realizar o levantamento fitossociológico das espécies vegetais do cerrado.
- Identificar as espécies selecionadas por ovinos em pastejo no cerrado.

- Selecionar espécies vegetais do cerrado, com eficácia para inibição de desenvolvimento larval.
- Avaliar, *in vitro*, a inibição do desenvolvimento larval de *H. contortus* de ovinos, sob a concentração de extratos de espécies vegetais do Cerrado.
- Determinar a DL 90 de dois extratos vegetais para a redução da eclosão de *H. contortus* de ovinos.
- Identificar e quantificar os compostos químicos presentes em espécies vegetais do Cerrado, com eficácia antihelmíntica.
- Testar a toxicidade das espécies vegetais selecionadas em camundongos.
- Avaliar os parâmetros sanguíneos em ovinos tratados com uma espécie do Cerrado.
- Testar, *in vivo*, a inibição do desenvolvimento larval de *H. contortus* de ovinos, sob a concentração de extratos vegetais do Cerrado.

CAPTER 1

Plants from Cerrado for the Control of Gastrointestinal Nematodes of Ruminants

Abstract: The gastrointestinal helminthes are major limiting factors for the sheep and goat production in the world and the health of livestock depends of effective control of nematodes. The constant administration and inadequate doses of chemical anthelmintics favors the selection of resistant populations and residues these products contribute to the contamination of animal products and of the ambient. The use of herbal treatment in veterinary medicine is a promising field of research. Studies in this area require the insertion into an agroecological context, with the limiting factor to the sustainable management of natural resources involved. The phytotherapy for the parasite control is an alternative that can reduce the cost with the purchase of anthelmintics as well, preventing the emergence of anthelmintic resistance and residues in animal products. Plant species that have tannins in its constitution are known to possess anthelmintic activity, requiring, however, that their efficacies are scientifically proven. The Cerrado is an import biome with high diversity of plants rich in tannins and other metabolic with potential anthelmintic effect. This study presents a review of research on plant species, tested in the Cerrado for the control of helminths in ruminants.

Keywords: anthelmintic, nematodes, medicinal plants, Cerrado, ruminants.

1 Introduction

The main problem in the small ruminants and limiting of economic exploitation is the gastrointestinal parasites. *Haemonchus contortus* is a nematode of abomasum and feeds of blood throughout, with high prevalence and high pathogenicity (Strong, 1993). Sheep with haemoncoses may show anemia and submandibular edema, with high mortality in young lambs and females in peripartum. Both sexes at all age levels may be intensely affected, reducing weight gain and reproductive capacity, as well as milk, wool, and hide production (Bizimenyera et al., 2006).

The treatment with anthelmintics has been intensely used to control by breeders. The constant administration and the inadequate dosages can favor the selection of the parasite populations resistant to the anthelmintics and contributes to the contamination of animal products with residues of these products (Amarante et al., 1992).

The main anthelmintics were developed during the 60's and are actually essential to control of nematodes. There are currently only three groups of broad spectrum anthelmintics and two groups of small spectrum used to control these parasites (Amarante et al., 1992). Early studies reported resistant helminthes to the group of benzimidazole and levamisoles. With the discovery of a chemical group distinct anthelmintic, avermectins, was represented an alternative treatment with a potent drug for the nematode control in domestic animals (Gopal et al., 1999). Multi-resistant nematodes have been found on several ruminant herds (Molento and Prichard 2001; Taylor et al., 2009; Wolstenholme et al., 2004; Thomaz-Soccol et al., 2004) . The possibility of anthelminthic residues in the environment and in animals reared for consumption (Hammond et al., 1997), as well as the spread of multi-resistant strains demands research into alternatives for gastrointestinal nematodes (GIN) control.

The utilization of plants containing secondary compounds such as condensed tannins may expand the organic alternatives to controlling GINs (Athanasiadou et al., 2007; Kahn and Diaz-Hernandez 2000). Phytotherapy in the control of parasitism is an alternative that can reduce the cost with the purchase of anthelmintics, and prevent the emergence of anthelmintic resistance and the presence of residues in animal products. Many plants are traditionally known as having anthelmintic activity, requiring, however, that their efficacy be scientifically proven (Vieira, 2003). Scientific validation of the anthelminthic effects and possible side-effects of plant products is necessary prior to their adoption as novel methods for control (Githiori et al., 2006).

The Cerrado biome, which covers 5% of the world flora, is the second largest source of biodiversity in Brazil (Sano, 2008). However, much of the native vegetation has been destroyed and many species are threatened of extinction, which would enable a wide use and maintenance of food, medicinal, ornamental, linseed and tannin production.

However, few studies have evaluated the anthelmintic effect of the plant species of the Cerrado for the control of GNI. Therefore, the analysis of potential plant species of this biome for helminthes control for ruminants may represent a promising strategy for the biotechnology industry and consequently for the breeders of these animals.

2 The Cerrado

Among the vegetation types that cover the American continent, the Cerrado presents a natural grandeur of plant species, which demonstrates the importance of education for the conservation and management of this biome. The original vegetation of the Cerrado has already been reduced by over 37 % (Felfili et al., 2002), prejudicing much of its biodiversity. Mittermeier et al. (1999) estimated that 67 % of the Cerrado areas are considered “highly modified” and only 20 % are in original condition, since the changes began with the colonization process, with the introduction of cattle, associated with rudimentary agricultural practices (Zanetti, 1994).

According Eiten (1993), the Cerrado’s flora is composed of two groups of species: thick stem trees and bushes with an undergrowth layer, consisting in a large mosaic, which includes a forest canopy formation more or less closed, containing trees with heights of 12 m tall or more. It has a woodland category, usually around six or seven meters and undergrowth stratum more or less continuous.

The herbaceous and shrub form a thick layer, especially grasses, making it difficult to distinguish individuals in both the layers as woodlands or as herbaceous due to many overhead structures being in accordance with shoots from the same root (Felfili et al., 2002).

The Cerrado *sensu strictu* is characterized by the presence of bent and twisted low trees; the shrub and herbaceous strata exhibits rapid growth during the rainy season (Ribeiro and Walter, 2008). These authors report that the Cerrado species have bent, twisted and gnarled timber trunks, leathery and rigid leaves, as adaptations to the dry environmental conditions. The most common species are represented by the Vochysiaceae and Fabaceae families, as well as species of Malpighiaceae, Anacardiaceae, Salicaceae, Rubiaceae (Felfili et al., 2002, Miranda et al., 2006), among others such as those represented by Caryocaraceae and Annonaceae families (Sales et al., 2009a; Sales et al., 2009b).

In regards climate, the average temperatures in the Cerrado areas vary between 22º C and 27 º C (Klink and Machado, 2005) , with average annual rainfall of 1,500 mm , water deficiency ranging from three to seven months of the year, depending on the region's seasonal (Nimer, 1989). The Cerrado's vegetation occurs predominantly in deep and well drained soil (Reatto et al., 1998), which present a lack of nutrients such as phosphorus and nitrogen , the pH being between 4.5 and 5.5, with high aluminum frequency rates (Ribeiro and Walter, 2008).

3 Anthelmintic Efficacy of Plant Species from Cerrado for Control of Gastrointestinal Nematodes

In recent years, society has prioritized environmental aspects, directing ample research towards the discovery of new bioactive substances that may be used in integrated pest management, with fewer negative effects on the environment (Castro, 1989).

In an attempt to contribute with an effective alternative control of gastrointestinal nematodes in small ruminants, several researchers have attempted to test plants used in folk medicine, evaluating the efficacy and safety of the same. Plant species rich in tannins called secondary metabolites have been extensively studied. The tannins anthelmintic action may act directly by interfering with the natural cycle of helminths, or indirectly, to protect the protein intake of ruminal degradation (with increased of protein availability in the lower gastrointestinal tract), which complicates the determination of its actual antiparasitic effect (Ketzis et al., 2006).

Furthermore, the results of *in vivo* tests conducted with these forages can be influenced by natural variations in the composition of the plant (by environmental factors or of their own cycle) that alter the concentration of the tannin intake by the animals (Athanasiadou and Kyriazakis, 2004).

The anthelmintic activity, attributed to tannins is present in plant species (Hoste et al., 2006). Calderon-Quintal et al. (2010) suggest that different strains of *H. contortus* show different sensitivities to the extracts rich in tannin and further studies are needed to confirm the *in vivo* results.

Chenopodium ambrosioides L. (Chenopodiaceae) “erva-de-santa-maria”, popularly known for its anthelmintic efficacy is a plant of the Chenopodiaceae family, with stem one meter tall with leaves shaped like spears with sinuous edges. The flowers are greenish, clustered in a small bouquet. From the leaves and flowers of this plant may be extracted an essential oil consisting of a mixture of mainly ascaridiol, silvestreno and safrole, and p-cymene and isohametina. The essential oil contains 60-80 % of ascaridiol with proven anthelmintic potential, abundant in the fruit, followed by flowers and leaves (Oliver-Bever, 1983).

Ketzis et al. (2002), working with essential oil of *C. ambrosioides* (0.2 mL/Kg^{-1} of body weight) achieved similar thiabendazole efficacy, promoting the impracticability of all the hatched larvae of *Haemonchus contortus* in sheep. However, Vieira (1992) noted no effect when administered infused orally to cattle.

The Annonaceae family includes about 50 genera and the genus *Annona* being one of the most important. The Annonaceae is characterized mainly by presenting a class of acetogenin substances. These substances are derived from long chain fatty acids, which act as potent inhibitor of mitochondrial respiration (Wang et al., 2002). The biological activities of *Annona* extracts have been attributed to the occurrence of annonaceous acetogenins, a class of natural compounds extracted from leaves (Geum-Soog et al., 1998; Wu et al., 1995) and seeds (Chang and Wu, 2001).

Annona squamosa L. (Annonaceae), known as the Earl fruit, “pinha” or “ata” are trees that can reach up to 5 m in height with long, thin and oval leaves. Its flowers have a greenish yellow color, adapting well to climates with little rain and with a well-defined dry season (Morton, 1987). Amorim et al. (1996) evaluated the aqueous extract from *A. squamosa* leaves, *in vitro*, on the first larval stages of gastrointestinal nematodes of cattle, obtaining mortality of 19.4 %. Vieira and Cavalcante (1999) tested *A. squamosa in vivo* on gastrointestinal nematodes in goats. The plant reduced by 40% the count of *H. contortus* eggs in feces. With respect to adult forms of the parasites, *A. squamosa* showed reduction rates in the population of *H. contortus* and *Trichostrongylus columbriformis* of 21.8 % and 31.4 %, respectively, however not reducing the *Strongyloides papillosus* population. Yet according to the authors, the extract showed still to be effective against the adult form of *Oesophagostomum columbianum*, reducing by 74 % the parasites. The acute toxicity of plants from the Annonacea family is still poorly studied and research approaches its *in vitro* cytotoxic efficacy and with possible emphasis on the anti-tumor effects (Vieira and Cavalcante, 1999).

Annona muricata L. (Annonaceae), popularly known as graviola, is a medium - sized fruit tree commonly found in the tropics. The species has been widely used in folk medicine as an anthelmintic, antipyretic, sedative, antispasmodic, and anticonvulsant and as a hypotensive agent in humans (Costa et al., 2002). *In vitro* tests to evaluate the inhibition of egg hatching, larval and adult worm motility are widely used in prospecting for new anthelmintic agents (Vasconcelos et al., 2007).

Ferreira et al. (2013), researching *H. contortus* in sheep, demonstrated that aqueous extract of *A. muricata* leaves at 50, 25, 12.5 and 6.25% concentrations inhibited larval hatching in 84, 9 , 79, 1, 66, 9 and 47.42 %, respectively. The authors also evaluated the effect on the motility of L3 *H. contortus* larvae at the same concentrations and obtained reduction motility rates at 83.29 %, 89.08 %, 74.62 % and

30.47 %, indicating significant activity of *A. muricata* on infective larvae of this parasite. However, when were evaluated the activity of the extract on the motility of adult parasites, the response was not dependent on dosage, being able to observe the extracts activity at different concentrations within the first six hours of exposure. Phytochemical analysis did not reveal any type of acetogenins or even alkaloids in the extract but indicated the presence of phenolic compounds in the aqueous leaf extract of *A. muricata* (Ferreira et al., 2013)

Furthermore, since acetogenins have been associated with neurodegeneration in rats and in humans (Champy et al., 2004) the absence of acetogenins in the *A. muricata* extract is a somewhat of a motivating fact, because it can make for this aqueous extract a safe drug to treat targeted animals if compared with plant extracts prepared with organic solvents presuming the extraction of acetogenin (Ferreira et al., 2013).

Annona crassiflora Mart. (Annonacea) commonly known as “panã”, “araticum”, “cabeça de negro” , “cascudo”, “cortiça”, “marolo” ou “pinha do cerrado”, stands out due to the fruit’s flavor, and is used in alternative medicine for possessing antibacterial and antifungal properties (Almeida et al., 1998). It is characterized by being a timber tree species, deciduous in the dry season, hermaphrodite and xerophytic. The phenology of this species is established by flowering early in the rainy season, which occurs from September to December, with fruiting having started in November, with ripened fruit from January to March (Lorenzi and Matos, 2002). The fruits are used as food and appreciated for having a sweet and yellowish pulp with a strong aroma (Roesler et al., 2007).

Queiroz et al. (2012), using ean thanol extract from the leaves of *A. crassiflora* verified the action of this extract on *H. contortus* larval development in sheep at 100 and 50 mg/mL^{-1} mg/mL^{-1}. The authors also obtained an anthelmintic efficacy superior to 98.6 % for the larval development of *H. contortus*, using dried leaves of the same plant at a coproculture concentration of 333.3 mg (ms)/mL^{-1}. The aqueous extract from the seeds and leaves of *A. crassiflora* showed anthelmintic efficacy of 99.43 % and 89.81 %, respectively at 100 mg/mL^{-1} (Nogueira, 2009), presenting a promising alternative for the control of *H. contortus* in sheep.

In Southeastern region of Brazil, and especially in the North and Northeast, the "cajazeira" (*Spondias mombin* L.) also known as "caja-mirim", "ambaró", "taperebá", is a fruit species belonging to the Anacardiaceae family. Utilized as source of permanent shading for the cocoa tree, it is also utilized by producing fruits that serve as an important source of additional income for the producer. The fruits' juicy, yellow, sour and aromatic properties are appreciated in refreshments and liquors (Sacramento, 2000).

The use of the "cajazeira" in folk medicine and by the pharmaceutical industry has increased, being utilized in the treatment of fevers, as an antidiarrheal, antidesintéric, antiblenorrágic and anti-hemorroidiary. According Sacramento (2000), research has recently revealed that the leaf extract contains ellagic tannins giving the plant antiviral properties. Ademola et al. (2005), using the *S. mombin* aqueous and ethanol extract against *H. contortus*, obtained a reduction of approximately 65% of eggs found in the sheep feces (OPG) at a 500 bw mg/Kg^{-1} concentration.

Lippia sidoides Cham. (Verbenaceae) or alecrim pimenta is a species often used as herbal medicine in Northeast of Brazil, due to the antiseptic action owing to the high levels of thymol and carvacrol (Matos and Oliveira, 1998). According Camurça-Vasconcelos et al. (2007) and Vasconcelos (2006), the essential oil of *L. sidoides* possess an inhibitory effect in vitro on *H. contortus* eggs in sheep at 0.02 mg/mL^{-1} to 1.25 mg/mL^{-1}, respectively. Souza et al. (2010), Bevillaqua et al. (2005), and Person (2001), obtained same results using this oil at 0.5% and 1%, respectively. In tests conducted *in vivo*, Camurça-Vasconcelos et al. (2008), reported an efficacy of 54% from the oil of *L. sidoides* in the control of *H. contortus* in sheep at a 283 mg/Kg^{-1}, 14 days after treatment.

The genus *Caryocar*, one of the representatives of the family Caryocaraceae family, has 16 species that are found in South and Central America (Maya et al., 2008). *Caryocar brasiliense* Cambess. specie is a tree species native to the Cerrado regions with wide distribution in the Southeast and Midwest of Brazil (Maia et al., 2008). The popular name of this plant species may vary according to the region of occurrence, the most common being: “Pequi”, “Piqui”,“piquiá-bravo”, “amêndoa de espinho”, “grão de cavalo”, “pequiá”, “pequiá-pedra”, “pequerim”, “Suari”and “piquiá” (Santos et al. 2004). Fruiting is annual and harvesting occurring in the period lasting from September to February (Vera et al., 2005).

The aqueous extract from the *C. brasiliense* fruit peels, at 200 mg/mℓ$^{-1}$, significantly inhibited the development of *H. contortus* larvae in sheep. The plant extracts effectiveness in the inhibition of larval development was of 94.8%. The egg-hatching inhibition of LC50 and LC90 was of 23.82 and 53.19 mg/mL^{-1}, respectively. The qualitative phytochemical tests performed in this study indicated the presence of catechins, steroids, flavonoids, saponins, total tannins, xanthones and tannins catechetical (Nery, 2009).

Nogueira et al. (2012) evaluated the aqueous extract of *C. brasiliense* fruit’s skin in the egg hatching inhibition test, with concentrations at 15 and 7.5 mg/ml^{-1}, reported anthelminthic efficacy corresponding to 98.7 % and 91.8 %, respectively. For these concentrations, the average L1 were significantly lower than treatment with distilled water or albendazole. The average for unembryonated eggs observed in all the treatments by extract was not different from the distilled water control and suggests that

"Pequi" metabolites do not inhibit the embryogenesis of these nematodes, while they may reduce hatching. The egg-hatching inhibition of LC50 and LC90 were 3.81 and 7.35 mg mL^{-1}, respectively.

In vivo, the average fecal egg count observed for the groups treated with the aqueous extract fruit peels of "Pequi" differed from the untreated group at concentration 2 g Kg^{-1} bw. During the first and second weeks of post treatment, it was observed a 33 and 32.2 % of anthelminthic efficacy *in vivo*, respectively, compared to pretreatment when all animals showed high levels of infection (Nogueira et al., 2012).

The crude powder derived from the "Pequi" fruit peels and leaves showed high efficiency (superior to 90 %) for the inhibition of larval development (LPGF) of *H. contortus* in sheep. The average LPGF for the concentrations at 250, 200 and 150 mg/mL were statistically similar to those observed for the control with the commercial anthelmintic. The aqueous extract from the leaves of the "Pequi" showed higher anthelmintic action within seven days of incubation. The lethal concentrations of LC50 and LC90 after seven days of incubation were 34.95 and 79.74 mg/mL, respectively, for the crude powder of the fruit peels and 69.05 and 97.19 mg/mL for the crude powder from the leaves. For the aqueous extract of the leaves, the LC50 and LC90 were 56.36 and 115.65 and mg mL^{-1}, respectively (Fonseca, 2012).

Morais-Costa et al. (2012) compared the efficacy of *C. brasiliense* from the northern and central region of Minas Gerais in Brazil. For both regions, the concentration 333.33 mg/mL^{-1} of dried leaves of *C. brasiliense* showed higher efficacy than negative control with distilled water and showed anthelmintic activity similar to the control with ivermectin (16 µ mL^{-1}). The dried leaves of this plant from northern and central region had anthelmintic action with efficacy of 98.52 % and 83.09 % respectively. This difference could be related to vegetation/area where the species were collected, since the area of vegetation in the northern region is a native and preserved area, which favors better performance and establishment of plant species in the Cerrado.

The species *Anacardium occidentale* L. belonging to the Anacardiaceae family, is popularly known as cashew tree (cajueiro). It is native to Brazil and used in traditional medicine, especially in northeastern Brazil due to its therapeutic effects. In the literature, there are proven pharmacological activities, as the cajueiro being anti-inflammatory plant (Olajide, 2004), ant diabetic (Barbosa-Filho et al., 2005), inhibitor of acetylcholinesterase (Barbosa-Filho et al., 2006) and antimicrobial (Akinpelu, 2001).

Aiming to evaluate the anti-parasitic activity of *Anacardium humile* A. St. - Hil. (Anacardiaceae). Nery et al. (2010), used aqueous and ethanolic extracts of leaves against different species of gastrointestinal nematodes in sheep. The aqueous extract anthelmintic activity showed significantly higher than negative control at all concentrations. At concentrations of 150 and 187.5 mg/mL^{-1}, the percent efficacy was not significantly different from ivermectin (positive control, 16 mg/mL^{-1}). The LD50 in the inhibition assay for larval development was 10.14 mg/mL^{-1}, and for the 5 % confidence interval it was 13.36-6.83 mg/mL^{-1}. Results of the ethanolic extract were not significantly different from ivermectin at 60 mg/mL^{-1}. The LD50 was mg/mL^{-1} 23.24. Larvae of *Haemonchus* spp. (68%), *Strongyloides* spp. (31%) and *Trichostrogylus* spp. (1%) were identified in the coprocultures of the negative control group. This suggests that the extracts were effective against the three nematodes considered to be the most prevalent and pathogenic in sheep (Ueno and Gonçalves, 1998).

Morais-Costa et al. (2012), in preliminary study, the activity of anthelmintic was evaluated to *Paullinea* sp. on gastrointestinal nematodes of sheep. The leaves of this plant were collected in the city of Montes Claros, Brazil. In this study, *Paullinea* sp. at 333.3 mg/mL^{-1} differed from the treatment with distilled water and showed anthelmintic activity of 70.12 %, similar to treatment with ivermectin. In the control group, 100% of larvae were *Haemonchus* sp.. The anthelmintic activity of the Sapindaceae family and the species *Paullinea* sp. may be associated with saponin and tannin respectively.

The “genipapo” (*Genipa americana* L.), Rubiaceae family, tree that has been used in folk medicine, foods and animal feed , leather tanning, forestry, and by logging industries. The species, native to South America, has ecological importance, and is suitable for planting in degraded areas and wetlands (Epistein, 2001). In the egg hatching inhibition test, the aqueous extract of *G. american* leaves at 100 mg/mL, completely inhibited hatching. The relative average number of embryonated eggs was significantly greater than those of unembryonated eggs at 75 and 100 mg/mL^{-1}. This observation suggests a greater efficacy in inhibiting hatching rather than interfering with embryo development. The LC50 and LC90 of aqueous extract from *G. American* leaves were 34.3 and 79.8 mg/mL^{-1}, respectively. In the larval development inhibition test, concentrations ≥ 30 mg/mL^{-1} showed anthelminthic efficacy above 94 %. The LC50 and LC90 mg/mL^{-1} were 14.6 and 28.7, respectively (Nery, 2009).

This suggests that the extracts were effective against the several nematodes considered to be the most prevalent and pathogenic in sheep (Wood et al., 1995), showing a wide spectrum of action. However, using the hydro-alcoholic extract from the leaves of “genipapo”, Krychak-Furtado (2006) found 100 % efficacy for EHI at 50 mg/mL^{-1}, thus suggesting the metabolites extracted with alcohol could also show action against nematode eggs.

In an experiment conducted by Costa (2010), the species *Schinopsis brasiliensis* Engl. (Anacardiaceae), *Baccharis tridentata* Vahl . (Asteraceae), *Ximenia americana* L. (Olacaceae), *Lippia sidoides* Cham. (Verbenaceae), *Paullinea* sp . (Sapindaceae) were selected by ruminants in the Cerrado, which are considered to be anthelmintic and tanniferous. The animals showed no worm problems during this research, but there is a need for *in vitro* and *in vivo* to better evaluate the effectiveness of these species.

It was reported by Morais-Costa et al. (2012) at a 333.3 mg/mL, the effectiveness of the dried leaves derived from the plant species *Evolvulus* sp. (Convolvulaceae), *Acosmium dasicarpum (*Vogel) (Fabaceae Faboideae) *Heteropterys byrsonymifolia* A. Juss. (Malphigiaceae), *Lippia sidoides* Cham. (Verbenaceae), *Erytroxylum deciduum* A.St.-Hil. (Erythroxylaceae), *Senna spectabilis* (DC.) HSIrwin & Barneby (Fabaceae), *Baccharis tridentata* Vahl. (Asteraceae), *Casearia sylvestris* Sw (Salicaceae), *Paullinea* sp. (Sapindaceae), *Piptadenia viridiflora* (Kunth) Benth (Fabaceae) and *Ximenia americana* L. (Olacaceae). After the logarithmic transformation and variance analysis, it was found that the average LDPG for treatments with the dehydrated leaves, did not differ from the control by ivermectin, and the efficacies were: *X. Americana* (99.84%), *P. viridiflora* (85.77%), *Paullinea* sp. (70.12%) and *C. sylvestris* (43.63%). This effect can be attributed to condensed tannin concentrations at 10 mg of ethanol extracts from *C. sylvestris* (7.36%) *Paullinea* sp. (6.37%), *P.viridiflora* (1.75%) and *X. Americana* (0.36%). This study demonstrates a potent anthelmintic activity *in vitro*, for the ethanol extract. The fact that some species of this study have low condensed tannin content highlights the synergism among chemical compounds.

4 Final Considerations

It is necessary to scientifically validate new alternative anthelmintic compounds, characterizing them in the control of ruminant GNIs, and to evaluate the toxicity of these compounds, *in vivo* experiments should be performed, providing some of the plant species to the animals. Thus, species rich in tannins, catechetic tannins, catechins, steroids, flavonoids, xanthones and saponins have promising potential in the control of nematodes of ruminants and furthermore, can be active in synergism of these metabolites.

The species *Anacardium occidentale*, *Annona crassiflora, A. muricata, A. squamosa*, *Caryocar brasiliensis, Chenopodium ambrosioides*, *Genipa americana Lippia sidoides, Paullinea* sp., *Piptadenia viridiflora, Spondias monbin and Ximenia Americana* are adapted to the Cerrado and showed very promising results in reducing the bioactivity in the development of gastrointestinal nematodes of ruminants in Brazil.

In order to clarify the mechanisms of action from extracts of plant species, on the development of larvae using an electronic microscopy, would be a tool to support the study on reducing the use of chemical products, favoring lower incidences of residues in products of animal origin, thereby reducing costs and environmental impacts of these products in the environment.

Plant species rich in tannins, saponins and other secondary compounds, are deserving of further accurate studies to prove the scientific efficacy in controlling gastrointestinal parasites. Therefore, few studies have evaluated the metabolic anthelmintic effect of plant species from the Cerrado as well as possible toxicity effects. Thus, emerges the need to use natural products, based on plant species that are naturally selected by ruminants in this biome.

References

Ademola, IO; Fagbemi, BO; Idowu, SO. Anthelmintis activity of extracts of *Spondias mombin* against gastrointestinal nematodes of sheep: studies in vitro and in vivo. *Tropical Animal Health and Production*, v. 37, n. 3, 223-35, 2005.

Akinpelu, DA. Antimicrobial activity of *Anacardium occidentale* bark. *Fitoterapia*, v. 72, 286-287, 2001.

Almeida, SP. *Frutas nativas do cerrado: caracterização físico-química e fonte potencial de nutrientes*. *In* Cerrado: ambiente e flora. Embrapa-CPAC, Planaltina, 1998, 247-281.

Amarante, AFT; Barbosa, MA; Oliveira, MAG; Carmello, MJ; Padovani, CR. Efeito da administração de oxfendazol, ivermectina e levamisol sobre os exames coproparasitológicos de ovinos. *Brazilian Journal of Veterinary Research and Animal Science*. São Paulo, v. 29, n. 1, 31-38, 1992.

Amorim, A; Rodrigues, MLA; Borba, HR. Influência de extratos vegetais *in vitro* na viabilidade de larvas de nematóides gastrointestinais de bovinos. *Revista Brasileira de Farmácia* , v. 77, 47-48, 1996.

Athanasiadou, S; Kyriazakis, I. Plant secondary metabolites: antiparasitic effects and their role in ruminant production systems. *Proceedings of the Nutrition Society*, v. 63, n. 4, 631-639, 2004.

Athanasiadou, S; Kyriazakis, I; Jackson, F; Coop, RL. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Veterirany Parasitology*, v. 99, 205-219. 2001.

Barbosa-Filho, JM; Medeiros, KCP; Diniz, MFFM; Batista, LM; Athayde-Filho, PF; Silva, MS; Da-Cunha, EVL; Almeida, JRGS; Quintans-Júnior, LJ. Natural products inhibitors of the enzyme acetylcholinesterase. *Revista Brasileira de Farmacognosia*, v. 16, 258-285, 2006.

Barbosa-Filho, JM; Vasconcelos, THC; Alencar, AA; Batista, L; Oliveira, RAG; Guedes, DN; Falcão, HS; Moura, MD; Diniz, MFFM; Modesto-Filho, J. Plants and their active constituents from South, Central, and North America with hypoglycemic activity. *Revista Brasileira de Farmacognosia*, v. 15, 392-413, 2005.

Bevilaqua, CML; et al. Ovicidal and larvicidal activity of *Lippia sidoides* and *Ocimum gratissimum* essencial oils against *Haemonchus contortus*. Proceedings oft the 20th International conference of the World Association for the Advancement of Veterinary Parasitology, Christchurch. *New Zealand Veterinary Journal*, v.1, 78-9, 2005. Disponível em: <http//www.sci quest.org.nz.pdf>. Acesso em: 26 agosto 2013.

Bizimenyera, ES; Githiori, JB; Eloff, JN; Swan, GE. In vitro activity of *Peltophorum africanum* Sond. (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *Trichostrongylus colubriformis*. *Vet Parasitol*, v. 142, 336-343, 2006.

Calderón-Quintal, JA; Torres-Acostaa, JFJ; Ca Sandoval Castroa, CA; Alonsob, MA; Hoste, H; Aguilar-Caballero, A. Adaptation of *Haemonchus contortus* to condensed tannins: can it be possible? *Arch Med Vet*, v. 42, 165-171, 2010.

Camurça-Vasconcelos, ALF; et al. Anthelmintic activity of *Lippia sidoides* essential oil on sheep gastrointestinal nematodes. *Veterinary Parasitology*, v. 154, 167-70, 2008.

Castro, AG. *Defensivos agrícolas como um fator ecológico*. Jaguariúna. EMBRAPA - CNPDA. Documento, 6, 1989.

Champy, P; Höglinger, GU; Feger, J; Gleye, C; Hocquemiller, R; Laurens, A; Guerineau, V; Laprevote, O; Medja, F; Lombes, A; Michel, PP; Lannuzel, A; Hirsch, EC; Ruberg, M. Annonacin, a lipophilic inhibitor of mitochondrial complex I, induces nigral and striatal neurodegeneration in rats: possible relevance for atypical parkinsonism in Guadeloupe. *J Neurochem*, v. 88, 63-69, 2004.

Chang, FR; Wu, YC. Novel cytotoxic annonaceus acetogenins from *Annona muricata*. *Journal of Natural Products*, v. 64, 925-931, 2001.

Costa, CTC; Morais, SM; Bevilaqua, CML; Souza, MMC; Leite, FKA. Ovicidal effect of *Mangifera indica* L. seeds extracts on *Haemonchus contortus*. *Brazilian Journal of Veterinary Parasitology*, v. 11, 57-60, 2002.

Costa, FM. Influência da estrutura da vegetação na seleção da dieta de ovinos em pastejo, em área de cerrado. Montes Claros, 2010. 78p. Dissertação (Mestrado). Universidade Federal de Minas Gerais/Instituto de Ciências Agrárias, 2010.

Eiten, G. Vegetação do cerrado. In: PINTO, M. N. (Org.). *Cerrado - caracterização, ocupação e perspectivas*. Brasília, DF. Editora da Universidade de Brasília, Brasília, 1993, 17-73.

Epistein, L. Cultivo e aproveitamento do jenipapo. *Rev Bahia Agrícola*, v. 4, 23-24, 2001.

Felfili, JM; Fagg, CW; Silva, JCS; Oliveira, ECL; Pinto, JRR; Silva-Júnior, MC; Ramos, KMO. *Plantas da APA Gama e Cabeça de Veado:* espécies, ecossistemas e recuperação. Brasília: Universidade de Brasília, DF. Departamento de Engenharia Florestal, 2002, 52 p.

Ferreira, LEA; Castro, PMN; Chagas, ACS; França, BSC; Beleboni, ARO. In vitro anthelmintic activity of aqueous leaf extract of *Annona muricata* L. (Annonaceae) against *Haemonchus contortus* from sheep. *Experimental Parasitology*, v. 134, 327-332. 2013.

Fonseca, LD. Potencial antihelmíntico de *Caryocar brasiliense* Cambess. (Caryocaraceae) no controle de nematódeos gastrintestinais de ruminantes. Montes Claros, 2013. 92p. Dissertação (Mestrado). Universidade Federal de Minas Gerais/Instituto de Ciências Agrárias, 2013.

Fortes, E. *Parasitologia veterinária*. Porto Alegre: Sulina, 606p., 1993.

Geum-Soog, K; Zeng, L; Alali, F; Rogers, LL; Wu, F; Mclaughlin, JL; Sastrodihardjo, S. Two new mono-tetrahydrofuran ring acetogenins, annomuricin E and muricapentocin, from the leaves of Annona muricata. *Journal of Natural Products*, v. 61, 432-436, 1998.

Gopal, RM; Pomroy, WE; West, DM. Resistance of field isolates of *Trichostrongylus colubriformis* and *Ostertagia circumcincta* to ivermectin. International. *Journal for Parasitology*, v. 29, 781-786, 1999.

Hammond, JA; Fielding, D; Bishop, SC. Prospects for plant anthelmintcs in tropical veterinary medicine. *Vet Res Commun*, v. 21, 213-228, 1997.

Hoste, H; Jackson, F; Athanasiadou, S; Thamsborg, SM; Hoskin, SO. The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends Parasitol*., v. 22, 253-261, 2006.

Kahn, LP; Diaz-Hernandez, A. *Nutrition: Proceedings of an international conference*. Canberra: Australian Centre for International Agricultural Research. Chapter 5, Tannins with anthelminthic, 2000, 130−139.

Ketzis, JK. et al. Evaluation of efficacy expectations for novel and non-chemical helminth control strategies in ruminants. *Veterinary Parasitology*, v.139, 321-335, 2006.

Ketzis, JK; Taylor, A; Bowman, DD; Brown, DL; Warnick, LD; Erb, HN. *Chenopodium ambrosioides* and its essential oil as treatments for *Haemonchus contortus* and mixed adult-nematode infections in goats. *Small Ruminantes Research*, v. 44, 193-200, 2002.

Klink, CA; Machado, RBA. conservação do cerrado brasileiro. *Megadiversidade*, Belo Horizonte, v. 1, 147-145, 2005.

Krychak-Furtado, S. Alternativas fitoterápicas para o controle da verminose ovina no estado do Paraná: testes in vitro e in vivo. 2006. 147 f. Tese (Doutorado em Agronomia) - Departamento de fitotecnia e fitossanitarismo. Universidade Federal do Paraná, Curitiba, 2006.

Lorenzi, H; Matos, FJA. *Plantas medicinais no Brasil:* nativas e exóticas cultivadas. Nova Odessa: Instituto Plantarum, 2002. v. 2.

Maia, JGS; Andrade, EHA; Silva, MHL. Aroma volatiles of pequi fruit (*Caryocar brasiliense* Camb.). *Journal of Food Composition and Analysis*, v. 21, 574-576, 2008.

Miranda, IS; Almeida, SS; Dantas, PJ. Florística e estrutura de comunidades arbóreas em cerrados de Rondônia, Brasil. *Acta Amazônica*, v. 36, 419-430, 2006.

Mittermeier, N; Myers, RA; Mittermeier, CG. *Hotspots*: earth's biologically richest and most endangered terrestrial ecoregions. Mexico: CEMEX, 1999, 430p.

Molento, MB; Prichard, RK. Effect of multidrug resistance modulators on the activity of ivermectin and moxidectin against selected strains of Haemonchus contortus infective larvae. *Pesq Vet Bras*, v. 21, 117-121, 2001.

Morais-Costa, F; Queiroz, IR; Vasconcelos; Ferreira, AVP; Costa, MAMS; Vieira, TM; Martins, MAD; Duarte, ER; Lima, WS. Efficacy of *Paullinea* sp. (SAPINDACEAE), in the alternative control of gastrointestinal nematodes, Minas Gerais state, Brazil. In: ATBC 2012. Bonito/MS., 2012.

Morais-Costa, F; Cruz, ALMC; Duarte, ER; Lima, WS. Potencial antihelmíntico de espécies vegetais do cerrado, na inibição do desenvolvimento larval de *Haemonchus* spp. In: Encontro de Parasitologia, 2012. Belo Horizonte/MG., 2012.

Morais-Costa, F; Queiroz, IR; Vasconcelos, VO; Fonseca, DL; Ferreira, AVP; Costa, MAMS; Vieira, TM; Mota, GS, Duarte, ER; Lima, WS. Eficácia de Caryocar brasiliense cambess. (Caryocaraceae) de diferentes regiões de minas gerais, no controle alternativo de nematódeos gastrintestinais. In: Congresso de Parasitologia, 2012, São Luiz/MA., 2012.

Morton, JF. Sugar Apple. In: *Fruits of warm climates*, 69-72, 1987.

Nery, PS. Eficácia de extratos vegetais no controle da helmintose ovina, no norte de minas gerais. Montes Claros, 2009. 102p. Dissertação (Mestrado). Universidade Federal de Minas Gerais/Instituto de Ciências Agrárias, 2009.

Nery, PS; Nogueira FA; Martins, ER, Duarte, ER. Effects of *Anacardium humile* leaf extracts on the development of gastrointestinal nematode larvae of sheep. *Veterinary Parasitology*, v.171, 361-364, 2010.

Nimer, E. *Climatologia do Brasil*. Rio de Janeiro: IBGE, 1989, 421 p.

Nogueira, FA, Nery, PS, Ferreira, M; Duarte, ER; Martins, ER. Plantas Medicinais no Controle Alternativo de Verminose em Ovinos. *Rev. Bras. de Agroecologia*, v. 4, n. 2, 2009.

Nogueira, FA; Fonseca, LD; Silva, RB; Ferreira, AVP; Nery, PS; Geraseev, LC; DuartE, ER. In vitro and in vivo efficacy of aqueous extract of *Caryocar brasiliense* Camb. to control gastrointestinal nematodes in sheep *Parasitol Res*, p. 111, 325-330, 2012.

Olajide OA. Effects of *Anacardium occidentale* stem bark extract on in vivo inflammatory models. *Journal of Ethnopharmacology*, v. 95, 139-142, 2004.

Oliver-Bever, B. *Medicinal plants in tropical west Africa*. *III Anti-infection therapy with higher plants*, v. 9, n. 1, 1983, 1- 85.

Queiroz, IR; Bastos, GA; Ferreira, AVP; Costa, FM; Duarte, ER; Oliveira, NJF. Eficácia de *Annona crasiflora* Mart. (Annonaceae) na inibição do desenvolvimeto larval de nematódeos gastroinstestinais de ovinos. In: XIV Semana da Biologia. 2012. Montes Claros/MG., 2012.

Queiroz, IR; Morais-Costa, F; Vascocelos, VO; Vieira, TM; Fonseca, LD; Ferreira, AVP; Costa, MAMS; Bastos, GA; Duarte, ER; Oliveira, NJFO. uso de *Annona crassiflora* (Annonaceae) no controle de nematóides gastrointestinais de ovinos. In: *Congresso de Parasitologia*. 2012. São Luiz/MA., 2012.

Reatto, A; Correia, JR; Spera, ST. Solos do bioma cerrado. In: SANO, S. M.; ALMEIDA, S. P. (Ed). *Cerrado*: ambiente e flora. Planaltina Embrapa/CPAC, 1998, 47-86.

Ribeiro, JF; Walter, BMT. As principais fitofisionomias do Bioma Cerrado. In: SANO, S. M.; ALMEIDA, S. P.; RIBEIRO, J. F. *Cerrado*: ecologia e flora. Brasília, DF, Embrapa Cerrados, 2008, 279 p.

Sacramento, CK; Souza, FX. de. *Cajá* (*Spondias mombin L*.). Jaboticabal: FUNEP, 2000, 42p.

Sales, HR; Santos, RM; Nunes, YRF; Morais-Costa, F; Souza, SCA. Caracterização florística de um fragmento de cerrado na APA Estadual do Rio Pandeiros – Bonito de Minas/MG. *MG. Biota*, Belo Horizonte, v. 2, 22-30. 2009.

Sales, HR; Souza, SCA; Luz, GR; Morais-Costa, F; Amaral, VB; Santos, RM; Veloso, MDM; Nunes, YRF. Flora arbórea de uma floresta estacional decidual na APA Estadual do Rio Pandeiros, Januária/MG. *MG. Biota*, Belo Horizonte, v. 2, 31-41, 2009.

Sano, SM; Almeida, SP; Ribeiro, JF. (Org.). *Cerrado*: ecologia e flora. Brasília-DF: Embrapa Informação Tecnológica, 2008, v. 2.

Santos, BR; Paiva, R; Dombroski, JLD; Martinotto, C; Nogueira, RC; Silva, AAN. *Pequizeiro (Caryocar brasiliense Camb.): uma espécie promissora do cerrado brasileiro*. Lavras: Editora Ufla, 2004 (Boletim Técnico).

Silva, GG; Souza, PA; Morais, PLD; Santos, EC; Moura, RD; Menezes, JB. Caracterização do fruto de ameixa silvestre (Ximenia americana L.). *Rev. Bras. Frutic.*, v. 30, 311-314, 2008.

Silva, RB; Rocha, FT; Morão, RP; Nogueira, FA; Marcelo, NA; Duarte, ER. Eficácia de levamizol e albendazol em rebanhos ovinos na região norte de Minas Gerais. In... 47ª Reunião Anual da Sociedade Brasileira de Zootecnia, 2010, Salvador, 2010.

Simões, CMO; Mentz, LA; Schenkel, EP; Irgang, BE; Stehmann, JR. *Plantas da Medicina Popular no Rio Grande do Sul*. *Porto Alegre*. 5º Edição. Ed. da Universidade/UFRGS, 1998, 42-5.

Souza, WMA; Ramos, RAN; Alves, LC; Coelho, MCOC; Maia, MBS. Avaliação *in vitro* do extrato hidroalcoólico (EHA) de alecrim pimenta (*Lippia sidoides* Cham.) sobre o desenvolvimento de ovos de nematódeos gastrointestinais (Trichostrongylidae). *Revista Brasileira de Plantas Medicinais*, Botucatu, v. 12, 278-281, 2010.

Taylor, MA; Learmount, J; Lunn, E; Morgan, C; Craig, BH. Multipleresistance to anthelmintics in sheep nematodes and comparison of methods used for their detection. *Small Ruminant Res*, p. 86, 67-70, 2009.

Thomaz-Soccol, V; Souza, FP; Cristina Sotomaior, C; Castro, EA; Milczewski, V; Mocelin, G; Pessoa E Silva, MC. Resistance of Gastrointestinal Nematodes to Anthelmintics in Sheep (*Ovis aries*) *Brazilian Archives of Biology and Technology*, v. 47, 41-47, 2004.

Ueno, H; Gonçalves, PC. *Manual para diagnóstico das helmintoses de ruminantes*. Tokyo. Japan International Cooperation Agency, Tokyo, 1998, p. 143.

Vasconcelos, ALC; Bevilaqua, CM; Morais, SM; Maciel, MV; Costa, CT; Macedo, IT; Oliveira, LM; Braga, RR; Silva, RA; Vieira, LS. Anthelmintic activity of *Croton zehntneri* and *Lippia sidoides* essential oils. *Veterinary Parasitology*, v. 148, 288-294, 2007.

Vasconcelos, ALCF. Avaliação da atividade antihelmíntica dos óleos essenciais de *Lippia sidoides* e *croton zehntneri* sobre nematoides gastrintestinais de ovinos. 2006. 83 f. Tese (Doutorado – Área de Concentração em Reprodução e Sanidade Animal) - Faculdade de Medicina Veterinária, Universidade Estadual do Ceará, Fortaleza, 2006.

Vera, R; Naves, RV; Nascimento, JL; Chaves, LJ; Leandro, WM; Souza, ERB. Caracterização física de frutos do pequizeiro (Caryocar brasiliense Camb.) no estado de Goiás. *Pesquisa Agropecuária Tropical*, v. 35, 71-79, 2005.

Vieira, LS. *Alternativas de Controle da Verminose Gastrintestinal dos Pequenos Ruminantes*. EMBRAPA, 10 p., 2003.

Vieira, LS; et al. Evaluation of anthelmintic efficacy of plants available in Ceará State, North East Brazil, for the control of goat gastrointestinal nematodes. *Revue de Medecine Veterinaire*, Toulouse, v.150, n. 5, 447-452, 1999.

Vieira, LS. *Fitoterapia da Amazônia: manual de plantas medicinais*. São Paulo: Agr. Ceres, 1992, 350 p.

Vieira, LS; Cavalcante, ACR; Pereira MF; Dantas, LB; Ximenes, LJF. Evaluation of anthelmintic efficacy of plants available in Ceará State, North-east Brazil, for the control of goat gastrointestinal nematodes. *Revue Médicine Véterinaire*, v. 150, 447-452, 1999.

Wang, Li-Quan; et al. Annonaceous acetogenins from the leaves of *Annona montana*. *Bioorganic & Medicinal Chemistry*, v.10, 561-565, 2002.

Wood, IB; Amaral, NK; Bairden, K; Duncan, JL; Kassai, T; Malone, JB; Pankavich Jr., JA; Reineche, RK; Slocombe, O; Taylor, SM; Vercruysse, J. World Association for the advancement of veterinary parasitology (W. A. A. V. P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). *Veterinary Parasitology*, v. 58, 181-213, 1995.

Wolstenholme, AJ; Fairweather, I; Prichard, RK; Von Samson-Himmelstjerna, G; Sangster, NC. Drug resistance in veterinary helminthes. *Trends Parasitol*., v. 20, 469-476, 2004.

Wu, FE; Zeng, L; Gu, ZM; Zhao, GX; Zhang, Y; Schwedler, JT; Mclaughlin, JL; Sastrodihardjo, S; New bioactive monotetrahydrofuran annonaceous acetogenins, annomuricin C and muricatocin C, from the leaves of *Annona muricata*. *Journal of Natural Products*, v. 58, 909-915, 1995.

Zanetti, R. *Análise fitossociológica e alternativas de manejo sustentável da mata da agronomia, Viçosa, Minas Gerais*. Viçosa, UFV. Trabalho integrante do conteúdo programático da disciplina Manejo Sustentado de Florestas Naturais, 1994, 92 p.

CAPTER 2

Plants of the Cerrado selected by grazing sheep with potential for inhibition of larva development of *Haemonchus contortus*

Abstract Plant species naturally selected by sheep grazing in the Cerrado region of Brazil were assessed for *in vitro* activity against *Haemonchus contortus.* After one year of observations, the plant families showing greatest richness in the region were Fabaceae, Rubiaceae, Malpighiaceae, Bignoniaceae, Myrtaceae and Annonaceae. Variation in the selectivity index for nine commonly plant species selected by grazing sheep was observed with respect to the dry and rainy seasons in the Cerrado. Coproculture was conducted in five replicates of 11 treatments: ivermectin, distilled water, or dehydrated leaves of nine selected plant species administered at 333.3 mg /g fecal culture. The dried powder of *Piptadenia viridiflora* and *Ximenia americana* leaves significantly reduced the number of infective larvae compared to the control with sterile distilled water. These species showed efficacy of over 85%, despite their low concentrations of proanthocyanidin. High-performance liquid chromatography analyses showed major peaks with UV spectra characteristic of flavonoids to extracts of these plants. Those naturally selected vegetal species with high anthelmintic efficacy show promise for use in diet as an alternative control of *Haemonchus contortus* in sheep.

Keywords Cerrado · vegetation structure · sheep grazing · phytochemical composition · Semiarid ·*Haemonchus contortus*

1 Introduction

The Cerrado is a region of savannah-type vegetation found in central Brazil, including the state of Minas Gerais. It is characterized by a high degree of diversity and endemism of species, accounting for 5% of the plant species on earth (Sano et al. 2008). Plant screening is recognized as a valuable strategy to identify new bioactive agents to develop new drugs for human or veterinary use.

Gastrointestinal nematode infections in sheep are a major cause of loss of production. *Haemonchus contortus* is the most prevalent and the most pathogenic species, being hematophagous in the abomasum (Arosemena et al. 1999). Infection can result in hemorrhagic anemia, dark-colored feces, edema, weakness, reduced production of wool and muscle mass, and sometimes sudden death (Taylor et al. 2007). Treatment with anthelmintics has been the only available measure to control haemonchosis (Paiva et al. 2001; Rangel et al. 2005). Their intensive use, often in sub-therapeutic doses associated with incorrect diagnosis, and the consistent use of a single pharmacological base, has favored the selection of multiresistant nematodes (Souza et al. 2008).

Plants extract can potentially be used as an alternative for reduction of costs and drug residues in animal products as well as to prevent the emergence of antihelmintic resistance. Some plants are traditionally believed to show antihelminthic activity, but their efficacy needs to be scientifically evaluated (Vatta et al. 2009).

Polyphenols, such as proanthocyanidins and tannins, are bioactive compounds found in several plant species that account for their pharmacological and nutraceutical properties. Antihelminthic activity of plants is credited to the presence of tannins (Hoste et al. 2006). To explore the potential of plants from the Cerrado for alternative helminthosis control it is necessary to conduct bioassays and quantify chemical constituents of the species, especially polyphenols.

The objectives of this study were to identify plant species naturally selected by sheep grazing in the Cerrado and to assess *in vitro* antihelminthic efficacy of these species on *Haemonchus contortus.*

2 Material and methods

2.1 Study area

The research was conducted from January 2009 through March 2010 in a rural area in Montes Claros City of North Minas Gerais state, Brazil (W 43º50’33.56”; S 16º41’10.05”). The climate of the region, tropical wet and dry (Aw) according to the Köppen classification, is marked by a long dry season from May to September and a rainy period in January and February. The monthly average rainfall and temperature during the dry season was 14.16 and 23.24ºC, respectively. In the rainy period, the monthly average rainfall and temperature was 201.32 mm and 24.32ºC. The area originally presents Cerrado vegetation, but actually is composed by patches of Cerrado in different stages of succession, due to the grazing of different domestic animals.

2.2 Collection and identification of plant species

The collection of tree, shrub, and herbaceous and trailing species in the area based on Müeller-Dombois and Ellenberg (1974) and Menino et al (2012). Identification was carried out according to morphological characteristics described by Lorenzi (2000,

2002) and Lorenzi and Matos (2002). Phytosociological parameters such as relative density, relative dominance, relative frequency, and importance value (IV) were calculated according to Müeller-Dombois and Ellenberg (1974).

2.3 Management of animal and plant species selected by sheep

Three adult male Santa Inês sheep of mean weight 35.4 kg were used. The animals were adapted to the study area and to the observers over the course of two months. They were released into the Cerrado for 3 h each morning and afternoon. All procedures were performed according to principles of animal experimentation approved by protocol 23/2009 by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais, Brazil.

The observations of plant selection were conducted after a 30 minute grazing adaptation period. For the verification of selected plants in the diet of three sheep, grazing was monitored on the first day of each month, observing the behavior for one hour in the morning and one hour in the afternoon. Three observers recorded the consumed plants every 5 minutes, totaling 12 observations daily for each animal. Considering the three animals, 432 observations were made over the course of one year (six observations/h x 2 h =12 observations per animal per month x 3 animals = 36 observations x 12 months = 432 observations). This method preserves animal welfare and allows good visualization of the selected vegetal species (Costa et al. 2009).

The calculation of selectivity index (SI) of plant species was adapted from the methodology used by Heady (1975), and consisted of the division of the percentage of selected and consumed plant species by the percent of those species in the area plant cover, multiplied by 100.

2.4 Dehydration and obtaining extracts of selected plant species

Healthy leaves were selected and dried to constant weight in a forced air circulating drier at 40ºC for 72 h. Dried leaves were ground and stored in paper bags in darkness. Aqueous extracts were obtained using the method of Nery et al. (2010) with modifications. Ground dried leaves were held in a distilled water bath at 40°C for 60 min, hot filtered through a gauze funnel, and the resulting extract was dehydrated at 40ºC for 48 h.

2.5 High-performance liquid chromatography (HPLC) analyses of the extracts

A Waters Alliance 2695 HPLC system composed of a quaternary pump, an autosampler, a photodiode array detector (DAD) 2996, and a Waters Empower Pro data handling system was used (Waters Corporation, Milford, USA). The analyses were performed on a LiChrospher 100 RP-18 column (250 × 4 mm i.d., 5 μm; Merck, Darmstadt, Germany) combined with a LiChrospher 100 RP-18 guard column (4 × 4 mm i.d., 5 μm; Merck) at 40 ºC. Water (A) and acetonitrile (B) were used as eluents, both containing 0.1% (v/v) of H_3PO_4 at a flow rate of 1.0 ml min^{-1} as follows: 0 min, 95% A and 5% B; 60 min, 5% A, 95% B, followed by 10 min of isocratic elution. Solvents used were of HPLC grade (Merck, Germany) and were degassed by sonication before use. The chromatograms were obtained at 210 nm, and the UV spectra were recorded on-line from 190 to 400 nm.

The dried aqueous extracts were dissolved in methanol (HPLC-grade), ultrapure water, or hydroethanolic solutions, according to their solubility, to concentrations of 10 mg /ml. After centrifugation at 8 400 x *g*, the sample solutions (10 μL) were automatically injected into the apparatus.

2.6 Spectrophotometric quantification of total proanthocyanidins

Total proanthocyanidin content of the dried aqueous extracts was determined by measuring at 540 nm the absorbance of the cyanidin chloride resulting from acid-catalyzed solvolysis with *n*-BuOH/HCl 37% (95:5), according to the method described by Hiermann et al. (1986). Each sample was analyzed in triplicate and the results expressed as mean ± standard deviation. The total proanthocyanidin content, expressed as cyanidin chloride, was calculated using the following formula:

$$Proanthocyanidin\ \% = \frac{Absorbance\ sample - Absorbance\ blank \times 4.155}{Weight\ sample\ (g)}$$

2.7 Inhibition of larva development

To evaluate the effectiveness of the dried leaves on larval development inhibition (LDI), the adapted coproculture quantitative methodology (Borges, 2003; Nery et al. 2010) was employed using feces of sheep with *Haemonchus contortus* mono-infection. All procedures performed were approved under protocol 25/2013 by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais, Brazil.

Eleven treatments were conducted, each with five replicates, including a positive control: 2 ml of solution 16 µg ml^{-1} ivermectin (LA Ranger, Vallée, Minas Gerais, Brazil) added to 2 g feces, and a negative control of 2 ml of sterile purified water added to 2 g feces. The nine plant treatments consisted of dehydrated and ground leaves of *Cerrado* species at final concentration of 333.3 mg of (dw) g^{-1} of feces culture. The materials were incubated in a BOD incubator at 28°C for seven days and assessed for presence of infective larvae (L3). The following formula, adapted from Borges (2003), was used to determine the percent reduction of larva /g of feces (LPGF):

% efficacy = 100 × (1 - LPGF of the treated group/LPGF of the treated group)

The data were log transformed, log (x+ 1) and submitted to variance analysis. The means were compared with the Duncan test at 5% probability using the SAEG 9.1 Program (2007).

3 Results

The area contained 1288 arboreal, 102 shrubs, and 1388 herbaceous species. A total of 94 plant species were grouped into 72 genera and 33 families. The phytosociology of 10 species arboreal, shrubs, and herbaceous species were ordered according to the IV values (Table 1). Voucher specimens were stored at the Montes Claros Herbarium (HMCMG) of Universidade Estadual de Montes Claros.

TABLE 1

Families and species recorded in vegetation of the Cerrado of North Minas Gerais, Brazil, with VN = voucher number; N = number of individuals; RD = relative density (%); Rdo = relative dominance (%); RF = relative frequency (%); and IV importance value (%)

Arboreal species	Family	N	RD	Rdo	RF	IV
Tachigali rugosa (Mart. ex Benth.) Zarucchi & Pipoly	Fabaceae	39	3.03	83.54	3.59	90.16
Heteropterys byrsonimifolia A.Juss.	Malpighiaceae	275	21.35	2.16	8.03	31.54
Astronium fraxinifolium Schott	Anacardiaceae	168	13.04	1.81	8.03	22.88
Machaerium opacum Vogel	Fabaceae	132	10.25	2.24	6.34	18.83
Copaifera langsdorffii Desf.	Fabaceae	87	6.75	2.81	4.23	13.79
Tabebuia aurea (Silva Manso) Benth & Hook.f. ex S.Moore	Bignoniaceae	65	5.05	0.83	5.07	10.95
Curatella americana L.	Dilleniaceae	55	4.27	1.22	3.38	8.87
Antonia ovata Pohl	Loganiaceae	47	3.65	0.65	4.23	8.53
Terminalia argentea Mart.	Combretaceae	41	3.18	0.49	4.44	8.11
Schwartzia adamantium (Cambess) Bedell ex Gir-Canãs	Marcgraviaceae	34	2.64	0.22	3.38	6.24
Shrubs species	Family	N	RD	Rdo	RF	IV

Lantana fucata Lindl.	Verbenaceae	14	13.73	6.75	11.54	32.02
Heteropterys byrsonimifolia A.Juss	Malpighiaceae	10	9.80	10.73	8.97	29.50
Astronium fraxinifolium Schott	Anacardiaceae	8	7.84	12.64	8.97	29.45
Tachigali rugosa (Mart. ex Benth.) Zarucchi & Pipoly	Fabaceae	12	11.76	6.47	8.97	27.20
Curatella americana L.	Dilleniaceae	4	3.92	6.10	5.13	15.15
Erythroxylum deciduum A.St.-Hil.	Erythroxylaceae	7	6.86	1.87	5.13	13.86
Guapira tomentosa (Casar.) Lundell	Nyctaginaceae	3	2.94	6.14	2.56	11.64
Byrsonima pachyphylla A. Juss.	*Malpighiaceae*	2	1.96	7.76	1.28	11.00
Cheiloclinium cognatum (Miers.) A.C.Sm.	Celastraceae	1	0.98	7.18	1.28	9.44
Banisteriopsis sp. 1	Malpighiaceae	3	2.94	3.66	2.56	9.16

Herbaceous species	Family	N	RD	Rdo	RF	IV
Evolvulus sp.	Convolvulaceae	507	36.52	36.41	35.8	108.73
Rhynchospora sp.	Cyperaceae	460	33.14	33.16	34.5	100.80
Hyptis sp.	Lamiaceae	131	9.43	9.46	10.7	29.59
Andropogon sp.	Poaceae	56	4.03	4.04	3.51	11.58
Zornia sp.	Fabaceae	48	3.45	3.46	4.29	11.20
Mascagnia sp.	Malpighiaceae	44	3.17	3.17	3.28	9.62
Stylosanthes sp.	Fabaceae	41	2.95	2.96	1.47	7.38
Chamaecrista sp.	Fabaceae	38	2.73	2.74	1.90	7.37
Coursetia sp.	Fabaceae	15	1.08	1.08	1.08	3.24
Belucia sp .	Melastomataceae	9	0.64	0.65	1.11	2.41

$RD_i = (RD_i / \sum (RD_i \ldots RD_n)) \times 100.$
$Rdo_i = (Rdo_i / \sum (Rdo_i \ldots Rdo_n)) \times 100.$
$RF_i = (RF_i / \sum (RF_i \ldots RF_n)) \times 100$

$IV = RD_i + RF_i + Rdo_i$

RD = indicates the percentage of each species of plant in relation to the whole plant community:
$RD_i = (RD_i / \sum (RD_i \ldots RD_n)) \times 100$.
So:
RDi =Relative density by species.

Rdo = allows calculating the percentage of dominance of each plant species in relation to the others:
$Rdo_i = (Rdo_i / \sum (Rdo_i \ldots Rdo_n)) \times 100$
So:
Rdo_i = relative dominance of species 'i'.

RF = Indicates the percentage frequency of plants of each species in relation to the plant community
$RF_i = (RF_i / \sum (RF_i \ldots RF_n)) \times 100$
So:
RF_i = relative frequency of the species 'i'.

After one year of monitoring, the selection of species was calculated from the selectivity index (SI) to determine the native plants most commonly consumed by grazing sheep. Considerable variation between the dry and rainy seasons was observed in these indices for nine species (Table 2).

TABLE 2

Selectivity index of the main plant species selected by grazing sheep in the Cerrado of North Minas Gerais, Brazil

Plants	2009									2010		
	Apr	May*	Jun*	Jul*	Aug*	Sep*	Oct	Nov	Dec	Jan*	Feb *	Mar
Baccharis cognata	10.74	0.00	11.57	29.57	0.00	0.00	8.10	0.00	0.00	0.00	0.00	0.00
Casearia sylvestris	4.75	0.00	23.00	5.23	36.72	40.01	3.58	7.38	3.10	3.52	0.00	3.42
Erythroxylum deciduum	2.80	3.43	1.88	0.77	3.31	0.55	0.79	4.90	0.00	3.52	14.77	6.84
Evolvulus sp.	4.05	5.26	4.91	3.56	3.03	2.79	1.55	4.57	5.38	7.42	7.32	4.03
Heteropterys byrsonimifolia	0.29	0.08	0.08	0.24	0.12	0.92	0.33	0.00	0.00	0.00	0.45	0.10
Paullinia sp.	29.15	0.00	0.00	0.00	12.53	30.72	0.00	0.00	9.52	32.47	0.00	0.00
Piptadenia viridiflora	0.00	0.00	0.00	2.61	0.00	0.00	0.00	0.00	0.00	0.00	3.69	0.00
Schinopsis brasiliensis	0.00	0.00	0.00	48.15	50.13	53.76	10.99	0.00	0.00	0.00	0.00	21.01
Ximenia Americana	0.00	0.00	0.00	2.34	0.00	0.00	16.03	13.23	0.00	6.31	3.31	3.06
Rainfall (mm)	64.9	5.45	0.00	0.00	0.20	48.4	323.9	135.8	219.5	27.8	17.3	262.5

* Dry period, rainfall < 50 mm

SI = Selectivity index: % consumed species / % species in the area × 100

Among nine species with selectivity values greater than 2.60%, five showed efficacy for inhibiting larva development in quantitative coprocultures. *Paullinia* sp. and *Piptadenia viridiflora* promoted significant reduction of the LDI average with efficacies of 71.7 and 86.5%, respectively (Table 3). *Ximenia americana* showed antihelminthic efficacy similar to that of the ivermectin treatment ($P < 0.05$, table 3).

TABLE 3

Mean numbers of infective *Haemonchus contortus* larvae in quantitative coproculture with powdered leaves of Cerrado plants naturally selected by sheep and the proanthocyanidin content in plant aqueous extract

Treatments	VN	LPGF **	efficacy (%)	Proanthocyanidin (% of dry matter ± s.d.)
Evolvulus sp.	928	1109.9 [a]	NE	NA
Heteropterys byrsonimifolia	2240	1026.0 [a]	NE	NA
Erythroxylum deciduum	2021	773.0 [a]	NE	NA
Baccharis cognata	3270	563.2 [a]	2.6	NA
Casearia sylvestris	3008	393.6 [a]	46.6	7.4 ± 0.1
Schinopsis brasiliensis	377	333.9 [a]	54.7	0.1 ± 0.4
Paullinia sp.	2249	208.7 [b]	71.7	6.4 ± 0.1
Piptadenia viridiflora	2283	99.4 [b]	86.5	0.2 ± 0.1
Ximenia americana	211	1.13 [c]	99.8	0.3 ± 0.1
Ivermectin (2.66 µg g^{-1})		3.0 [c]	99.6	NA
Distilled water		737.6 [a]		

Means followed by different letters in the columns indicate significant differences ($P < 0.05$), by Duncan test. Coefficient of variation LPGF: 12.64%
*VN: Voucher number
** LPGF: number of larvae (L3) per gram of feces

Efficacy: % efficacy = 100 x (1 - LPGF of the treated group/LPGF of the treated group).
NA: not assayed (below the limit of quantitation of the method)
NE: Not effective

The chemical composition of the selected species was acessed by their contents of proanthocyanidins, quantified using spectrophotometry, as well as by the chromatographic profiles registered by HPLC-DAD. *Ximenia america* and *Piptadenia viridiflora* showed efficacy of over 85%, but presented low concentrations of proanthocyanidin (Table 3). The occurrence of polyphenols in all active species is suggested by their HPLC-DAD chromatograms, which indicated the predominance of peaks corresponding to polar compounds, with UV spectra compatible with polyphenols (Fig. 1).High-performance liquid chromatography analyses showed major peaks with UV spectra characteristic of flavonoids to extracts of *P. viridiflora* (λ 262.7 and 376.8 nm) and *X. america* (λ 255.6 and 348.3 nm) (Fig. 2).

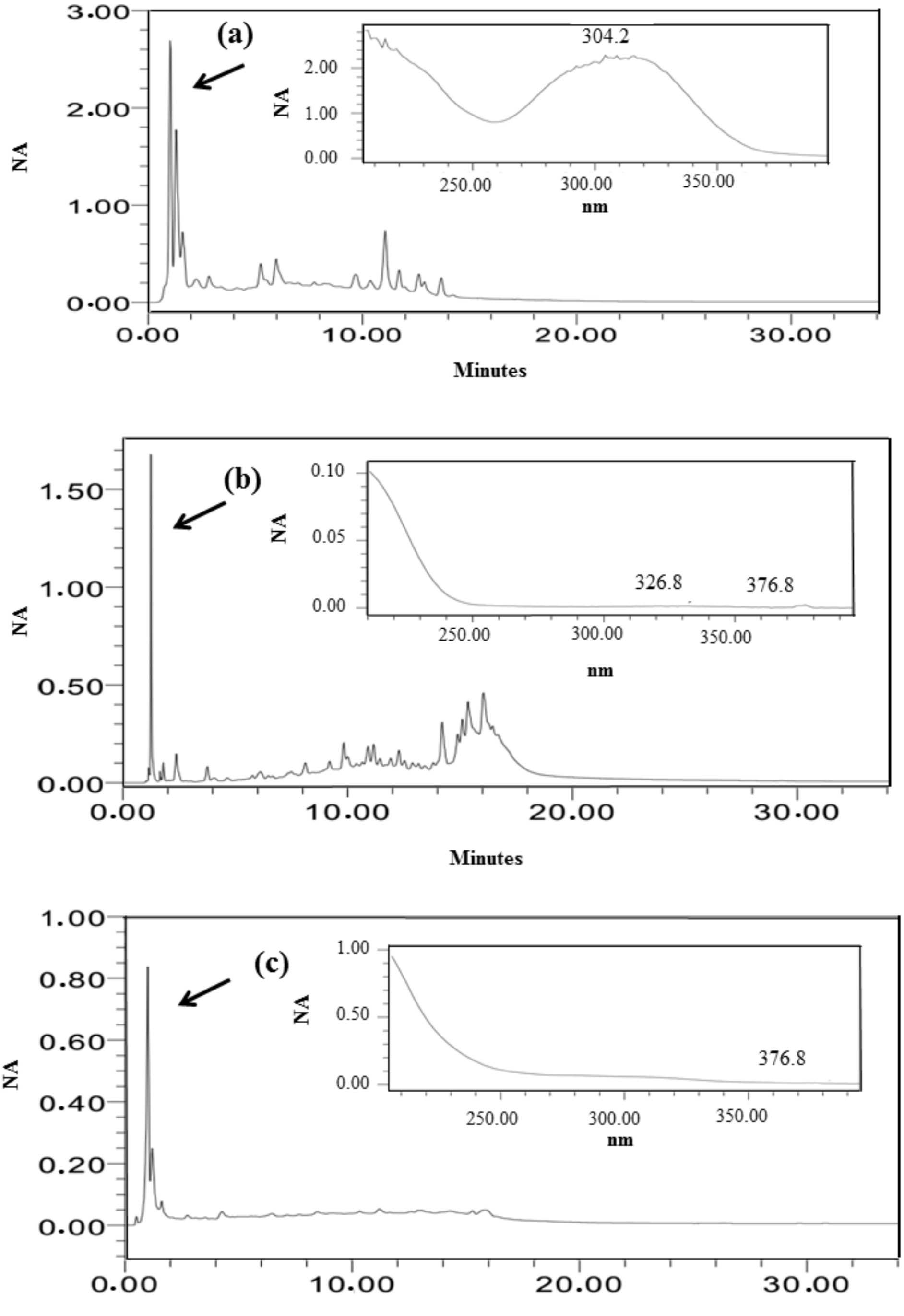


Fig. 1 HPLC-DAD chromatograms obtained for the aqueous extracts of the species active in the antihelminthic assay. (a) *Casearia sylvestris*, (b) *Schinopsis brasiliensis* and (c) *Paullinia* sp.

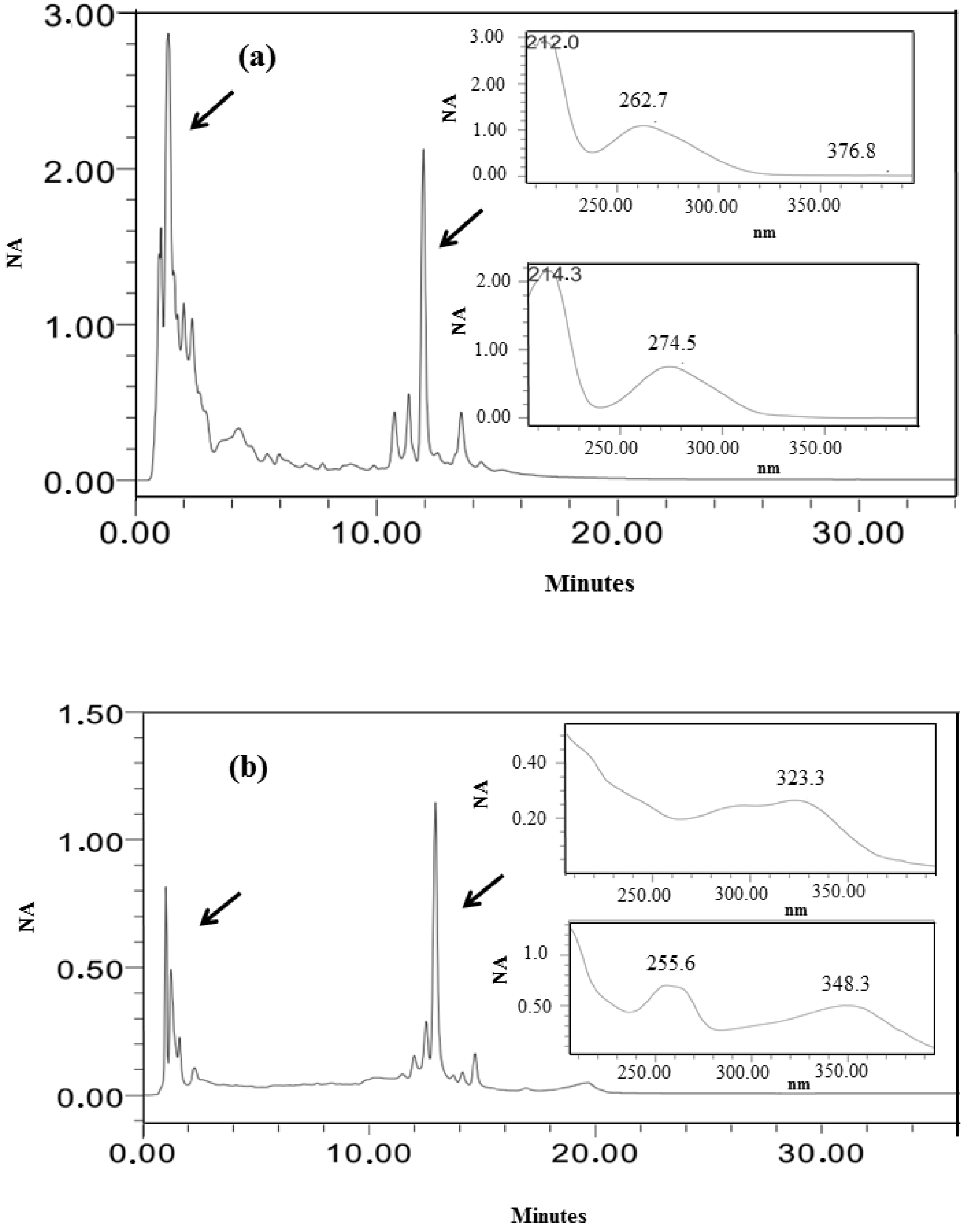


Fig. 2 HPLC-DAD chromatograms obtained for the aqueous extracts of the species active in the antihelminthic assay. (a) *Piptadenia viridiflora*, and (b) *Ximenia americana*.

4 Discussion

The plant families showing greatest richness were Fabaceae with 15 species, Rubiaceae and Malpighiaceae, with eight, Bignoniaceae and Myrtaceae, with seven, and Annonaceae with four (Table 1). These families were comparable to those reported by Cerrado of Miranda et al. (2006), Sales et al. (2009a, b) and Menino et al. (2012).

The absolute values of selectivity index were based on a scale having the central point value of one, indicating the balance between the percentage of a species selected by the grazing animal and the percentage of that plant in the cover of the grazing area. An index of less than one indicates that a species was seldom selected. An index greater than one indicates a higher level of selection (Santos et al. 2008).

The commonly selected species *Erytroxylum deciduum, Astronium fraxinifolium, Machaerium opacum, Tabebuia aurea, Copaifera langsdorffii, Senna spectabilis, Ximenia americana*, and *Schinopsis brasiliensis* (Table 2) were also identified by Sales et al. (2009a, b) in other Cerrado areas.

Baccharis cognata, *Casearia sylvestris*, *Paullinia* sp., and *Schinopsis brasiliensis* showed higher SI during the dry season (Table 2). This period is critical for animal feeding, because the supply of forage is compromised in quantity and quality.

Assessment of nutritive value and toxicological analyzes may show a potential of these plants for supplementation of the diet of sheep and for a role of grazing on these plants in the strategic control of nematode infection, which is commonly concentrated in the dry season (Niezen et al. 2002; Taylor et al. 2007). *Evolvulus* sp., *Erytroxylum deciduum*, and *Heteropterys byrsonimifolia* were selected throughout the evaluation period (Table 2), indicating the potential for use in both dry and rainy seasons.

Paullinia sp. and *Piptadenia viridiflora* and *Ximenia americana* promoted significant reduction of the LDI average with efficacies more than 70% (Table 3). *Casearia sylvestris*, and *Paullinia* sp. showed the highest contents of proanthocyanidins but were not among those with the highest anthelmintic efficacy *in vitro* (Table 3).

Piptadenia viridiflora was selected only in July 2009 and February 2010. February represented the end of the rainy season, and the end of new growth. Possibly in this period *P. viridiflora* was not harmful to sheep, although it has been reported to be toxic at 4.43 g kg / bw (Tocarnia et al. 1999). This species contains hydrocyanic acid bonded to carbohydrates called cyanogenic glycosides that is released upon hydrolysis (Vetter, 2000).

Ximenia americana was selected by the animals in January 2010, in the plant fruiting period. The seeds are considered purgative (Pio Correa 1984). The species has been shown to exhibit healing properties, which can be credited to the presence of tannins (Monte et al. 2012). In general, the compounds found in *X. americana* were saponins, glycosides, flavonoids, tannins, phenolics, alkaloids, quinines, and terpenoids. In addition, the plant is rich in fatty acids and glycerides, and the seeds contain cyanide derivatives (Monte et al. 2012).

Among the five active species, only *C. sylvestris* and *Paullinia* sp. presented a high content of condensed tannins, suggesting that other compounds may account for the antihelminthic properties of *S. brasiliensis*, *P. viridiflora*, and *X. americana*. HPLC chromatograms of *P. viridiflora* and *X. americana*, species with low proanthocyanidin content present major peaks with UV spectra characteristic of flavonoids (Fig. 2).

Tannins can exert direct antihelminthic action, interfering with the natural cycle of helminths, or indirectly, by inhibiting the degradation of ruminal protein (Ketzis et al. 2006). Some Cerrado species possessing these compounds have been shown to inhibit larval development of gastrointestinal nematodes in ruminants (Paolini et al. 2005; Oliveira et al. 2011; Nery et al. 2010**;** Nogueira et al. 2012; Ferreira et al. 2013; Cala et al. 2014).

The aqueous extract of *Caryocar brasiliense* fruit specie occurring in the Cerrado biome, peel tested at 200 mg /ml , significantly inhibited the development of *H. contortus* larvae in sheep (Nogueira et al. 2012). *In vitro* tests of extracts of this plant for inhibition of larval development showed 94.8% effectiveness (Nogueira et al. 2012). The qualitative phytochemical tests performed indicated the presence of catechins, steroids, flavonoids, saponins, xanthones, and tannins (Nogueira et al. 2012).

In another study, the aqueous extract of seeds and leaves of *Annona crassiflora* species occurring in the Cerrado biome showed antihelminthic efficacy of 99.43 % for seeds and 89.81% for leaves at 100 mg /mlin LDI with quantitative coproculture (Nogueira et al. 2009). Nery et al. (2010) evaluated extracts of leaves of *Anacardium humile* A. St.-Hil (Anacardiaceae) against several species of gastrointestinal nematodes in sheep. The aqueous extract showed antihelminthic activity significantly higher than that of the negative control at all concentrations evaluated. At 150 and 187.5 mg/ml, the percent efficacy was not significantly different from ivermectin at 16 mg /ml . Larvae of *Haemonchus* spp. (68%), *Strongyloides* spp. (31%), and *Trichostrogylus* spp. (1%) were identified in the coprocultures of the negative control group. This extracts were effective against the three nematodes most prevalent and pathogenic in sheep (Wood et al. 1995).

Variation in the selectivity index for nine main plant species selected by grazing sheep was observed with respect to the dry and rainy seasons in the Cerrado. The dried leaves of *C. sylvestris, S. brasiliensis, Paullinia* sp., *P. viridiflora*, and *X. americana* show LDI efficacy ranging from 46.6% to 99.6%. Despite having low levels of condensed tannins, *P. viridiflora* and *X. americana* showed higher antihelminthic efficacy than did other selected plants and are promising for use in diet and alternative control of *Haemonchus contortus* infections in sheep.

5 Statement of Animal Rights

All procedures were performed according to principles of animal experimentation approved by protocol 23/2009 by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais, Brazil.

References

Arosemena, N.A.E., Bevilaqua, C.M.L., Melo, A.C.F.L., Girão, M.D. 1999. Seasonal variations of gastrointestinal nematodes in sheep and goats from semiarid area in Brazil. Revue De Medicine Veterinaire. 150, 873–876.

Borges, C.C.L. 2003. Atividade *in vitro* de anti–helmínticos sobre larvas infectantes de nematódeos gastrintestinais de caprinos, utilizando a técnica de coprocultura quantitativa (Ueno, 1995). Parasitologia Latinoamericana. 58, 142–147.

Costa, F.M., Geraseev, L.C., Martins, E.R., Jayme, D.G. 2009. Metodologias de determinação da composição botânica da dieta de ruminantes em áreas de vegetação nativa. Caderno de Ciências Agrárias. 1, 86–96.

Ferreira, L.E.A., Castro, P.M.N., Chagas, A.C.S., França, B.S.C., Beleboni, A.R.O. 2013. *In vitro* anthelmintic activity of aqueous leaf extract of *Annona muricata* L. (Annonaceae) against *Haemonchus contortus* from sheep. Experimental Parasitolology. 134, 327–332.

Heady, H.F. 1975. Range Management. McGraw–Hill Book Company, New York, USA, pp. 1–460.

Hiermann, A., Kartnig, T.H., Azzam, S. 1986. Ein Beitrag zur quantitativen Bestimmung der Procyanidine in *Crataegus*. Scientia Pharmaceutica. 54, 331–337.

Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S. M., Hoskin, S. O. 2006. The effects of tannin–rich plants on parasitic nematodes in ruminants. Trends in Parasitolology. 22, 253–261.

Ketzis, J.K., Vercruysse, J., Stromberg, B.E., Larsen, M., Athanasiadou, S., Houdijk, J.G.M. 2006. Evaluation of efficacy expectations for novel and non–chemical helminth control strategies in ruminants. Veterinary Parasitology. 139, 321–335.

Lorenzi, H. Brazilian trees. 2002. A Guide is the Identification and Cultivation of Arboreal Plants Native of Brazil. Second Ed. Plantarum, Nova Odessa, SP, BR, pp. 17–383.

Lorenzi, H. Plantas daninhas do Brasil: terrestres, aquáticas, parasitas e tóxicas. 2000. Terceira Ed. Plantarum, Nova Odessa, SP, BR, pp. 7–640.

Lorenzi H.; Matos, F. J. A. 2002. Plantas medicinais no Brasil: nativas e exóticas cultivadas. 2002. Segunda Ed. Nova Odessa: Plantarum, Nova Odessa, SP, BR, pp. 11–544.

Menino, G.C.O., Nunes, Y.R.F., Santos, R.M., Fernandes, G.W., Fernandes, L.A. 2012. Environmental heterogeneity and natural regeneration in riparian vegetation of the brazilian semi–arid region. Edinburgh Journal of Botany. 69, 29–51.

Miranda, I.S., Almeida, S.S., Dantas, P.J. 2006. Florística e estrutura de comunidades arbóreas em cerrados de Rondônia, Brasil. Acta Amazonica. 36, 419–430.

Monte, F.J., Lemos, T.L.G., Araújo, M.R.S., Gomes, E.S. 2012. *Ximenia americana*: Chemistry, Pharmacology and Biological Properties, a Review. In: Phytochemicals – A Global Perspective of Their Rolein Nutrition and Health, Venketeshwer Rao, Rijeka, HRV, pp. 429–450.

Müller–Dombois, D., Ellenberg, H. 1974. Aims and methods in vegetation ecology. In: The Count–Plot method and Plotes Sampling Techniques, John Wiley and Sons, Nova Jersey, EUA, pp. 93–135.

Nery, P.S., Nogueira, F.A., Martins, E.R., Duarte, E.R. 2010. Effect of *Anacardium humile* on the larval development of gastrintestinal nematodes of sheep. Veterinary Parasitology. 171, 361–364.

Niezen, J.H., Robertson, H.A., Sidey, A., Wilson, S.R. 2002. The effect of pasture species on parasitism and performance of lambs grazing one of three grass–white clover pasture swards. Veterinary Parasitology. 105, 303–315.

Nogueira, F.A., Silva, P.N., Souza, M.F., Duarte, E. R., Martins, E.R. 2009. Plantas Medicinais no Controle Alternativo de Verminose em Ovinos. Revista Brasileira de Agroecologia. 4, 2353–2356.

Nogueira, F.A., Fonseca, L.D., Silva, R.B., Ferreira, A.V.P., Nery, P.S., Geraseev, L. C., Duarte, E.R. 2012. *In vitro* and *in vivo* efficacy of aqueous extract of *Caryocar brasiliense* Camb. to control gastrointestinal nematodes in sheep. Parasitology Research. 111, 325–330.

Paiva, F., Sato, M.O., Acuña, A.H., Jensen, J.R., Bressan, M.C.R.V. 2001. Resistência a ivermectina constatada em *Haemonchus placei* e *Cooperia punctata* em bovinos. A Hora Veterinária. 20, 29–32.

Paolini, V., Prevot, F., Dorchies, Ph., Hoste, H. 2005. Lack of effects of quebracho and sainfoin hay on incoming third–stage larvae of *Haemonchus contortus* in goats. The Veterinary Journal. 170, 260–263.

Pio Correa, M. 1984. Dicionário das plantas úteis do Brasil e das exóticas cultivadas. In: Serviço de Informação Agrícola. Terceira Ed. Ministério da Agricultura. Rio de Janeiro, BR, pp. 543–544.

Rangel, V.B., Leite, R.C., Oliveira, P.R., Santos Júnior, E.J. 2005. Resistência de *Cooperia* spp e *Haemonchus* spp as avermectinas em bovinos de corte. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 57, 186–190.

Saeg. 2007. Sistema para Análises Estatísticas, Versão 9.1. Viçosa: Fundação Arthur Bernardes – UFV.

Sales, H.R., Santos, R.M., Nunes, Y.R.F., Morais–Costa, F., Souza, S.C.A. 2009a. Caracterização florística de um fragmento de cerrado na APA Estadual do Rio Pandeiros – Bonito de Minas/MG. MG. Biota. 2, 22–30.

Sales, H.R., Souza, S.C.A., Luz, G.R., Morais–Costa, F., Amaral, V.B., Santos, R.M., Veloso, M.D.M., Nunes, Y.R.F. 2009b. Flora arbórea de uma floresta estacional decidual na APA Estadual do Rio Pandeiros, Januária/MG. MG. Biota. 2, 31–41.

Sano, E.E., Rosa, R., Brito, J.L.S., Ferreira, L.G. 2008. Mapeamento semidetalhado do uso da terra do bioma Cerrado. Pesquisa Agropecuária Brasileira. 43, 153–156.

Santos, G.R.A., Batista, A.M.V., Guim, A., Santos, M.V.F., Silva, M.J.A., Pereira, V.L.A. 2008. Determinação da composição botânica da dieta de ovinos em pastejo na Caatinga. Revista Brasileira de Zootecnia. 37, 1876–1883.

Souza, A.P., Ramos, C.I., Bellato, V., Sartor, A.A., Schelbauer, C.A. 2008. Resistência de helmintos gastrintestinais de bovinos a anti–helmínticos no Planalto Catarinense. Ciência Rural. 38, 1363–1367.

Taylor, M.A, Coop, R.L., Wall, R.L. 2007. Veterinary Parasitology. In:Parasites of sheep and goats. Third Ed. Blackwell Publishing, Oxford, UK, pp. 133–136.

Vatta, A.F., Waller, P.J., Githiori, J.B., Medley, G.F. 2009. The potential to control *Haemonchus contortus* in indigenous South African goats with copper oxide wire particles. Veterinary Parasitology 162, 306–313.

Vetter, J. 2000. Plant cyanogenic glycosides. Toxicon. 38, 11–36.

Wood, I.B., Amaral, N.K., Bairden, K., Duncan, J.L., Kassai, T., Malone, J.B., Pankavich, J.A., Reinecke, R.K., Slocombe, O., Taylor, S.M., Vercruysse, J. 1995. World Association for the Advancement of Veterinary Parasitology WAAVP second edition of guidelines for evaluating the efficacy of anthelminthics in ruminants bovine, ovine, caprine. Veterinary Parasitology. 58,181−213.

CAPTER 3

***Piptadenia viridiflora* (Kunth) Benth selected from Cerrado to control of *Haemonchus contortus* in lambs**

Abstract

Resistance to anthelminthics has been common in different continents. *Piptadenia viridiflora* is an plant from Cerrado selected naturally by sheep and could be source of bioactive compounds for development of new farmacs. In this study, *in vitro* and *in vivo* efficacy this vegetal was evaluated for lambs naturally infected with *Haemonchus contortus*. The presence of tannins and flavonoids for aqueous and ethanolic extracts of the leaves was indicated by HPLC-DAD. These extracts at 2.4 and 1.2 mg mL^{-1}, respectively, showed 100% efficacy to egg hatch inhibition (EHI). In larval development inhibition (LDI) test with quantitative cultures, the aqueous extract ≥ 1.2 mg mL^{-1} promoted L3 mean lower than those observed for the control with water ($P<0.05$) and the estimated LC90 was 2.28 mg g^{-1} of feces. Maximum tolerated dose for male and female mice was > 12.87 mg kg^{-1} bw in intraperitoneal via. Aqueous extract was orally administered at 283 mg (dm) kg^{-1} bw during three consecutive days and the anthelminthic efficacies observed up to three weeks pos-treatment were between 32.9 - 47.2% with fecal egg counts (FEC) lower averages than untreated lambs ($p<0.05$). For all sheep erythrocyte and hematocrit values were within the normal physiological patterns and plasmatic albumin and total protein were similar between *in vivo* treatment groups. Low concentrations of extracts promoted high efficacy for EHI and LDI. The oral treatment with aqueous extract is promising since shows moderated *in vivo* anthelminthic efficacies, not interference with normal clinical standards and no toxicity to blood parameters evaluated.

Keywords: Brasilian savanna, sheep, antihelminthic plant, phytochemical composition, toxicity, blood parameters.

1 Introduction

Haemonchus contortus is parasite of the abomasum and responsible for most of losses in the sheep creations. Lambs with haemonchosis can show anemia and submandibular swelling, with high mortality in young lambs and females in peripartum. Both sexes at all age levels may be intensely infected, reducing weight gain and reproductive capacity, as well as milk, wool, and hide production (Bizimenyera et al. 2006).

Anthelmintics have quick solution for the control of this nematode, but resistance have been observed in diferente countries such as New Zealand (Leathwick et al. 2001), Switzerland (Schnyder et al. 2005), Italy (Cringoli et al. 2007), Africa (Soro et al. 2013), Nigeria (Adiele, et al. 2013) and Brazil (Araújo e Lima, 2005; Duarte et. al., 2012).

The constant administration and the inadequate dosages can favor selection of the populations resistant to the anthelmintics (Cooper et al. 2011; Power et al. 2013) and contributes to the contamination of animal products with residues of these products and environment (Power et al. 2013). The utilization of plants containing secondary compounds such as condensed tannins has represented organic alternatives to controlling for gastrointestinal nematodes (Athanasiadou et al. 2004).

Therefore, the analysis of potential plant species fron Cerrado biome for helminthe control can represent promising strategy for the biotechnology industry and consequently for the breeders (Nogueira et al., 2012; Nery et al. 2010; Iqbal 2005).

Piptadenia viridiflora (Kunth) Benth. (Fabaceae) it is popularly known as “surucucu” (Lorenzi, 2009), is found frequentily in the Cerrado (Fig. 1) and shows, has medicinal properties with compounds such as tannins and flavonoids (Lorenzi, Matos, 2002).

Figure 1 – *Piptadenia viridiflora* (Fabaceae)

However species contains hydrocyanic acid bonded to carbohydrates called cyanogenic glycosides that is released after hydrolysis (Vetter, 2000), which may be toxic if used in an amount above 9 g kg / bw (Tokarnia, et al. 1999).

In prelimary study of Cerrado Biome, *P. viridiflora* was naturally selected by lambs grazing during the dry and rainy seasons, with selectivity index 2.61% and 3.69% respectively (Morais-Costa, et al., 2014), when this ratio is ≥ one indicates that this vegetal species is important in the animal's diet . Then the purpose in this study was to analyze *in vitro* and *in vivo* extracts of *Piptadenia viridiflora* to control *Haemonchus contortus*.

2 Material and methods

2.1 Study area

The research was conducted in a rural area of Montes Claros of North of Minas Gerais state, Brazil (W 43º50'33.56"; S 16º41'10.05"). The climate of this region is tropical wet and dry (Aw) according to the Köppen classification and marked by long dry season from May to October and rainy period in November to April.

2.2 Production of vegetal extratcts

Healthy leaves of *P. viridiflora* from Cerrado Biome were selected and dried to constant weight in a forced air circulating drier at 40ºC for 72 h. Dried leaves were grinded in a wiley mill and then were stored in paper bags, free of light incidence. Samples of the plant were stored in the Montes Claros Herbarium (HMCMG) of the Universidade Estadual de Montes Claros-Brazil, with voucher specime, 2283.

Aqueous extract of dried leaves were held in a distilled water bath at 40°C for 60 min. Ethanolic extract was obtained by maceration of the dried leaves in absolute ethyl alcohol (PA), in glass amber containers, kept in a dark place and stored for seven days. After this extraction, filtration was held through a gauze funnel. The extracts were dehydrated at 40ºC for 48 h, until obtaining the residue with constant weight and stored in paper bags in darkness and refrigerated at ∼4ºC until use (Adapted Nery et al., 2010).

2.3 High-performance liquid chromatography (HPLC) analyses and proanthocyanidin quantification.

A Waters Alliance 2695 HPLC system composed of a quaternary pump, an autosampler, a photodiode array detector (DAD) 2996, and a Waters Empower Pro data handling system was used (Waters Corporation, Milford, USA). The analyses were performed on a LiChrospher 100 RP-18 column (250 × 4 mm i.d., 5 µm; Merck, Darmstadt, Germany) combined with a LiChrospher 100 RP-18 guard column (4 × 4 mm i.d., 5 µm; Merck) at 40 ºC. Water (A) and acetonitrile (B) were used as eluents, both containing 0.1% (v/v) of H_3PO_4 at a flow rate of 1.0 mL min^{-1} as follows: 0 min, 95% A and 5% B; 60 min, 5% A, 95% B, followed by 10 min of isocratic elution. Solvents used were of HPLC grade (Merck, Germany) and were degassed by sonication before use. The chromatograms were obtained at 210 nm, and the UV spectra were recorded on-line from 190 to 400 nm.

The dried aqueous and ethanolic extracts were dissolved in methanol (HPLC-grade), ultrapure water, or hydroethanolic solutions, according to their solubility, to concentrations of 10 mg mL^{-1}. After centrifugation at 8 400 x *g*, the sample solutions (10 µL) were automatically injected into the apparatus.

Total proanthocyanidin content of the dried aqueous extracts was determined by measuring at 540 nm the absorbance of the cyanidin chloride resulting from acid-catalyzed solvolysis with *n*-BuOH/HCl 37% (95:5), according to the method described by Hiermann et al. (1986). Each sample was analyzed in triplicate and the results expressed as mean ± standard deviation. The total proanthocyanidin content, expressed as cyanidin chloride, was calculated using the following formula:

$$\% = \frac{A_{\text{sample}} - A_{\text{blank}} \times 4.115}{m_{\text{sample}}}$$

A is the measured absorbance at 420 nm, and m_{sample} is the sample weight in g.

2.4 Egg hatching inhibition (EHI)

The aqueous and ethanolic extract of the dried leaves was at 4.8 mg mL^{-1} and diluted in sterile distilled water. These extracts were used in EHI tests immediately after dissolution as described Coles et al. (1992).

Flotation, sedimentation, and filtration techniques in saturated NaCl solution were conducted to obtain nematode eggs from feces of two Santa Inês lambs infected only with *Haemonchus contortus* and with an average fecal egg count (FEC) >1000 g^{-1}, determined using the modified McMaster technique (Gordon and Whitlock, 1939).

Experimental mixtures contained: 100 µl fecal suspension with an average of 80 hanging eggs, and 100 µl of the extract, at final concentrations 0.15-2.4 mg mL^{-1} or two positives controls with solution levamisole phosphate (0.3 mg mL^{-1}) or ivermectin (16 µ mL^{-1}) or a negative control with sterile distilled water. The samples were homogenized and incubated in a BOD incubator at 28°C for 48 h. Subsequently, 15 µl Lugol's solution was added to each tube, which were then stored at 4°C for subsequent counting of unembryonated eggs, embryonated eggs, first stage larvae (L1; Coles et al. 1992).

The number of L1 relative to the total number of eggs plus L1 was determined for each repetition and subjected to variance analysis. The means were compared using the Tukey test at 5% significance. Probit regression was employed to determine the concentrations sufficient to inhibit 90% (lethal concentration, LC90) of egg hatching using the statistical package, Saeg 9.1 (2007).

The formula of Coles et al. (1992) was used to determine the EHI effectiveness:

% effectiveness = 100 x (1 – mean of L1 / mean eggs eggs + L1)

2.5 *In vitro* larval development inhibition (LDI)

The effectiveness of the aqueous extract was evaluated by adapted coproculture quantitative methodology (Borges, 2003; Nery et al. 2010, Nogueira, 2012) using fresh feces of lambs with *Haemonchus contortus* mono-infection**.**

Nine treatments were performed, each with five replicates, including two positive control: 2 mL of solution 16 µg g^{-1} ivermectin (final concentration) or 2 mL of solution 0.1 mg g^{-1} levamisole phosphate (final concentration) and a negative control of 2 mL of sterile purified water, both added to 2 g feces.

The six treatments with *P. viridiflora* was standardized at 1.21-38.62 final concentration mg of dw g^{-1} of fecal culture. The samples were incubated in a BOD incubator at 28°C for seven days and assessed for presence of infective larvae (L3). The following formula, adapted from Borges (2003), was used to determine the percent reduction of larva g^{-1} of feces (LPGF):

% efficacy = 100 × (1 - LPGF of the treated group/LPGF of the treated group)

The data were transformed in log (x + 1) and submitted to variance analysis. The means were compared through the Duncan test at 5% probability and LC_{90} was determined by probit analysis using the statical package SAEG® 9.1 (2007).

2.6 Toxicity in mice

The mouse toxicity testing was performed as Walum (1998) to determine the maximum tolerated dose (MTD) for an adult mouse. Using probe gavage 22µL of aqueous extract of *P. viridiflora* was added for four Balb C mice (2 males and 2 females) with average 22g of body weight and 6 to 8 weeks.

For the first and second days the extract was diluted at 100 and 10 times respectively in 1x PBS. In the third and fourth days the extract was administered at 38.62 mg ml^{-1}. The mice were euthanized by cervical dislocation on day 5 after extract administration.

2.7 *In vivo* anthielmintic test

The analyses were performed on 24 Santa Inês lambs with average 26.5 kg bw of both sexes, with 4-to-8-month-old. Prior to the beginning of the trial, all sheeps were administered albendazole (10 mg kg^{-1} bw) and phosphate levamisole (0.6 mg kg^{-1} bw), to ensure that were worm-free.

During 10 days of adaptation, the animals were individually confined which fed balanced diet containing sorghum silage, concentrate, mineral premix and water *ad libitum*, according to the age category requirement.

The animals, with zero fecal egg count (FEC), were infected with 800 L3 for $10kg^{-1}$ bw from lambs naturally contaminated in *H. contortus*. Twenty-eight days post-infection, sheep were assigned to one of three homogeneous groups based on FEC, weight, and sex.

One untreated animals served as the negative control, other group was orally administered phosphate levamisole (0.6 mg kg^{-1} bw) via subcutaneous and represented the positive control, the third group was administered aqueous vegetal extract of *P. viridiflora*, by esophageal gavage, at 283 mg (dm) kg^{-1} bw during three consecutive days.

The mean of two counts of FEC was obtained for each collection periods. The McMaster technique was utilized with the addition of saturated NaCl for FEC in duplicate (Gordon and Whitlock, 1939).

Initial FEC value was recorded based on the mean values of three days prior to initiation of treatment. Subsequently, mean FEC was determined for three periods post-treatment to efficacy analysis: 7, 8 and 9 days (first period), 14, 15 and 16 (second period) and 21, 22 and 23 (third period). Fecal samples were cultured to obtain larvae for identification and confirmation of mono-infection by *H. contortus* (Ueno & Gonçalves, 1998).

The data relating FEC values were previously transformed into log (x + 10) and subjected to analysis of variance and the means were compared by Duncan test ($p<0.05$). A formula adapted from Coles et al. (1992) was used to determine the percentage efficacy in FEC reduction:

Efficacy = 100 x (1 – mean FEC of treated group/mean FEC of control group)

2.8 Blood parameters of sheep

Blood samples were collected from jugular vein on days 0, 7, 14 and 21 into tubes containing (EDTA) and transported at 4°C. Erythrocytes and hematocrit were evaluated in an automatic analyzer (2.800 BC Vet®, Mindray Medical International Ltd. in Shenzhen, China). The protein and albumin values were analyzed in enzymatic kits (Bioclin ® -. Quibasa Basic Chemicals Ltd., Belo Horizonte, MG, Brazil) by colorimetric spectrophotometer (Automatic system for biochemical - BIOPLUS BIO 2000, Shenzhen, China). Data were subjected to variance analysis with split plots.

Means were compared by the Scott-Knott test at 5% probability using the SAEG 9.1 package.

All procedures were performed in accordance with the principles of animal experiments approved in the 275/2013 protocol of the Ethics Committee on the use of animals (CEUA) of the Federal University of Minas Gerais, Brazil.

3 Results and discussion

3.1 Extracts characterization

The presence of polyphenols for both extracts was indicated by HPLC-DAD chromatograms, which clearly indicate the predominance of peaks corresponding to polar compounds, with UV spectra compatible with polyphenols (Fig. 2).

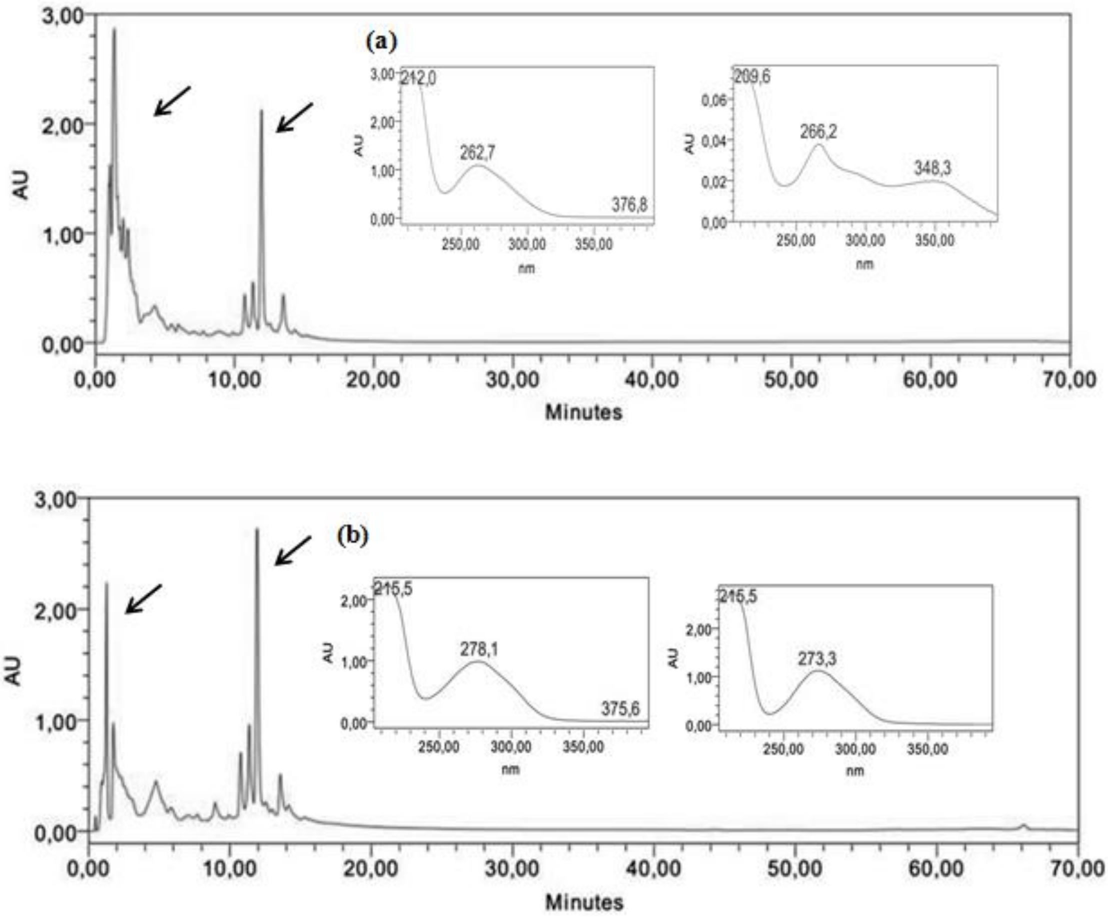


Figure 2 - HPLC-DAD chromatogram obtained for the aqueous (a) and ethanolic (b) extract of the dried leaves of the *P. viridiflora* in the antihelminthic assay. Chromatographic conditions: see Experimental section.

In addition, the HPLC chromatograms both extracts showed present major peaks with UV spectra characteristic of flavonoids (λ 262.7 and 376.8 nm aqueous and λ 278.1 and 375.6 to ethanolic). The quantification total de proanthocyanidin concentrations for aqueous and ethanolic extracts were 0.23% ± 0.2 and 1.75% ± 0.3 respectively. It showed that proanthocyanidin from *P. viridiflora* was better extracted by ethanol.

Flavonoids and tannins can present beneficial effects in animals infected with gastrointestinal nematodes (Barrau et al., 2005) and has been used to improve the growth performance of animals (Niezen et al 1998, Athanasiadou et al 2001, Paolini et al 2004, Valderrábano et al 2010).

This study showed *P. viridiflora* with low content of condensed tannin when compared to other Cerrado species *Ouratea semiserrata* (6.63%) and *Ouratea spectabilis* (9.99%) (Valadares et al., 2003). Thus other compounds of *P. viridiflora* as flavonoids could be acting in the control of *H. contortus.*

Between Cerrado species with anthelmintic effects, phytochemical tests indicated the presence of tannins and flavonoids for *Anacardium humile* (Nery et al. 2010) and for *Cariocar brasiliense* were identified condensaded tannins, hydrolysable tannins, flavonoids, terpenoids (Bezerra et al. 2002; Paula-Junior et al. 2006), and saponins (Paula-Junior et al. 2006). Flavonoids also were observed to aqueous extract of immature mango that showed *in vitro* and *in vivo* inhibition of *Haemonchus contortus* (Camurça-Vasconcelos et al. 2007, Nogueira et al. 2012).

3.2 Egg hatching inhibition

The effectiveness was directly related with increasing concentration of the aqueous extract of *P. viridiflora* leaves. The aqueous and ethanolic efficacies were at 13.16-100% and 69.6-100% respectively (Table 1), and LC_{90} for these extracts were 2.62 and 2.70 mg mL^{-1} respectively.

All concentrations tested for both extracts promoted significant reduction for hatched larvae mean when compared to the negative control with distilled water. The aqueous extracts showed greater inhibition of larval hatching, whereas ethanol extract showed the best inhibition of early embryonic development (Table 1). These differences could be attributed to the higher concentration of proanthocyanidin in the ethanol extract (1.75%) compared to aqueous (0.23%).The extract treatments promoted embryonated eggs with degraded larvae and lesions to cuticle of L1 (Fig. 3a, b).

Table 1- Aqueous and ethanolic extracts of *Piptadenia viridiflora* leaves in egg hatching of *Haemonchus contortus*

Treatments	Unembryonated egg mean	Embryonated egg mean	L1 mean	Eggs + L1	Efficacy* (%)
Aqueous extract (mg mL^{-1})					
2.4	7.2^{b}	35.8^{b}	0.0^{c}	35.8	100.0
1.2	2.0^{b}	43.8^{ab}	1.6^{c}	45.4	98.08
0.6	2.5^{b}	48.8^{a}	20.2^{c}	69.0	75.84
0.3	1.4^{b}	42.2^{ab}	46.0^{b}	88.2	44.98
0.15	1.4^{b}	13.0^{c}	72.6^{a}	85.6	13.16
Variation coefficient (%)	59.44	22.60	42.98		
Ethanolic extract (mg mL^{-1})					
1.2	36.0^{bc}	0.0^{b}	0.0^{b}	36.0	100.0
0.6	49.0^{bc}	0.0^{b}	7.6^{b}	56.6	90.9
0.3	54.2^{abc}	0.0^{b}	11.4^{b}	65.6	86.4
0.15	49.8^{bc}	4.0^{b}	21.6^{b}	75.4	74.2
0.075	25.2^{cd}	0.0^{b}	25.4^{b}	50.6	69.6
L. phosphate (0.3 mg mL^{-1})	78.0^{ab}	0.0^{a}	2.6^{b}	80.6	100.0
Ivermectin (16µ mL^{-1})	78.8^{a}	0.0^{a}	0.0^{b}	0.0	96.88
Sterile distilled water	0.0^{d}	0.0^{ab}	83.6^{a}	83.6	___
Variation coefficient (%)	30.12	119.39	76.45		

Mean followed by a different letter in the columns indicates significant differences by tukey test ($P < 0.05$)
*% efficacy = 100 x (1 - L1/initial egg + L1).

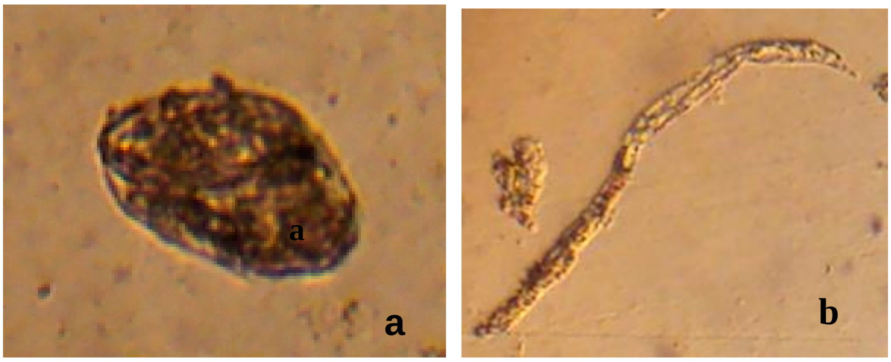


Fig. 3 - *Haemonchus contortus* with embryonated egg degradation (a) and L1 with cuticle alteration (b) by the action of leaves ethanolic extract of *Piptadenia viridiflora* (objective 10×)

Research with other Cerrado plants has also reported high efficacies in EHI tests. The aqueous extract from fruit's skin of *Caryocar brasiliense,* popularly named as "pequi, in the EHI test at 15 mg ml^{-1} presented anthelminthic efficacy of 98.7 and LC_{90} was 7.35 mg ml^{-1} (Nogueira et al. 2012a). Aqueous extract of *Annona muricata* at 50% inhibited 84.91% the egg hatching of *H. contortus* (Ferreira et al., 2013).

3.3 Larval development inhibition

The aqueous extract ≥ 1.2 mg mL^{-1} showed mean of L3 significantly lower than those observed for the control with water (P<0.05). There was increase to effectiveness of LDI with increased concentration (Table 2) and estimated LC90 was 2.28 mg g^{-1} of feces.

Table 2 - Mean *Haemonchus contortus* larvae per gram of feces (LDPG) in quantitative cultures treated whit different concentrations of the aqueous extract of the *Piptadenia viridiflora* leaves

Treatments	LPGF*	Efficacy (%)
Aqueous extract (mg g^{-1})		
38.62	0 [f]	100.00
19.31	55 [e]	93.13
9.65	105 [d]	86.88
4.83	180 [c]	77.05
2.41	285 [b]	64.38
1.21	385 [b]	55.63
Levamisole phosphate (0.1 mg g^{-1})	0 [f]	100.00
Ivermectin (16µ mL^{-1})	0 [f]	100.00
Sterile distilled water	800 [a]	---

Means followed by different letters in the columns indicate significant differences (P< 0.05), by Duncan test.
Coefficient of variation LDI: 4.67%
*LPGF: number of larvae (L3) per gram of feces
Efficacy: % efficacy = 100 x (1 - LPGF of the treated group/LPGF of the treated group).

In other research with Cerrado species has reported LDI efficacies at higher concentration than *P. viridiflora.* The aqueous extract from *Caryocar brasiliense* fruit peels at 200 mg mL^{-1} significantly inhibited the development of *H. contortus* larvae from sheep with efficacy of 94.8%. The qualitative phytochemical tests performed indicated the presence of catechins, steroids, flavonoids, saponins , xanthones and tannins that could promoted mixed actuation (Nogueira et al. 2012). Ferreira et al. (2013), demonstrated that aqueous extract of *Annona muricata* (Panã) leaves at 50% concentration inhibited larval hatching in 83.29%. Nery et al. (2010), evaluated extracts of leaves *Anacardium humile* A. St.-Hil. (Anacardiaceae), against different species of gastrointestinal nematodes in sheep. The aqueous extract at 150 mg mL^{-1} showed LDI significantly higher than negative control at all concentrations evaluated.

In other study, the aqueous extract of immature fruits at 50.0 mg ml^{-1} showed effective anthelminthic activity for LDI of 90 % and the fresh juice of mango showed 100 % efficacy at 59.2 mg ml^{-1} (Nery et al. 2012).

3.4 Toxicity test

For evaluated concentrations, no clinical signs of toxicity for mucosal tissues or changes in animal behavior or deaths during were observed in the four days of extract administration. During the autopsy, macroscopical alterations were no observed in the liver, kidneys, spleen, lungs or other viscera. Then maximum tolerated dose for male and female mice was > 38.62 mg kg^{-1} bw.

3.5 *In vivo* anthelmintic activity

The FEC was influenced by treatments and periods. Oral administration of *P. viridiflora* extract promoted reduction of FEC means for the three pos-treatments periods that was significantly lower than the observed for the control group in its respective periods. The *in vivo* anthelminthic efficacies were between 32.9 - 47.2% (Table 3) and the animals showed no behavioral changes, submandibular edema, weakness or lack of appetite during the experiment.

Table 3 - Mean of fecal egg count (FEC) from sheep and anthelminthic efficacy after oral administration of aqueous extract of the *P. viridiflora* leave at 283 mg (dm) kg^{-1} bw or levamisole phosphate at 0.6 mg kg^{-1} bw

Treatments	Initial period	First week		Second week		Third week	
		FEC g^{-1}	Efficacy (%)	FEC g^{-1}	Efficacy (%)	FEC g^{-1}	Efficacy (%)
Control	5601,75 A	3950,0 Aa	--	2675 Ba	--	2650 Ba	--
Levamisole	4483,00 A	66,7 Bc	99.99	29,25 Cc	98.90	18,75 Dc	97.48
P. viridiflora	5962,37 A	2088,0 Bb	47.15	1469,00 Cb	45.10	1781,00 Cb	32.86

Means followed by different uppercase letters in rows and lowercase letters in columns differ by Scott-Knott test with values of $P<0.05$

Efficacy = 100 x (1 – mean FEC of treated group/mean FEC of control group)

Variation coefficient of = 8.85%

The aqueous extract of *P. viridiflora* leaves produced lower reduction when compared to synthetic anthelminthics recommended by World Association for the Advancement of Veterinary Parasitology (WAAVP). However these results are promising, since it was used at low doses and administered in three single doses and could reduce multi-resistant nematode populations. Future studies with a larger dose at greater frequency may indicate better anthelminthic efficacy *in vivo*.

Other researches with plants from Cerrado have reported *in vivo* efficacies similar with the observed in this study. *Pterocaulon interruptum* aqueous extract were administered orally at 33.34 mg kg^{-1} bw reduced to 47% of the FEC in sheeps (Krychak-Furtado, 2006). Significant FEC reductions were observed in lambs treated with the "traditional" of the dried bark *Albizia anthelmintica* - Fabaceae (Coração-de-nego) preparation with efficacy of 34% (Githiori et al., 2003).

In sheep naturally infected with mixed species of gastrointestinal nematodes in the Pakistan was administered aqueous methanolic extract of *Caesalpinia crista* (L) seed and *Chenopodium album* (L) whole plan, the maximum FEC reductions were and 93.9 and 82.2% with *C. album* and *C. crista* at 3.0 g kg^{-1} bw, on day 13 and 5 post-treatment, respectively.

In experiments *in vivo*, the extracts of plants often do not reach the level proposed for commercial anthelminthic products by the WAAVP (Githiori et al. 2006). In this study was verified high *in vitro* efficacies e a lower *in vivo* efficiency. Differences of anthelminthic efficacies of plant treatment between *in vitro* and *in vivo* tests have been reported (Peneluc et al., 2009, Nogueira et al., 2012). Report that the efficacy of the plant extracts may vary with bioavailability of plant compounds in different compartments of the gastrointestinal tract of ruminates infected and with the parasite specie, producing divergent results study between *in vitro* and *in vivo* with the same plant species (Athanasiadou et al., 2007; Eguale et al., 2007).

3.6 Blood parameters of sheep

Erythrocyte and hematocrit values were within the normal physiological patterns for lambs (Table 4). The hematocrit is good indication of the anemia level and represents important parameter for the assessment hematophagous parasites (Sotomaior et al. 2007). In cases of acute haemonchosis, anemia is characterized by progressive decrease in hematocrit (Taylor et al. 2010). Values below 15% are concomitant with weakness and indicate bad prognosis for the animal (Bowman 2010). Costa et al. (2011) found hematocrit values ranging from 24.7 to 29.6%, respectively, for the treated sheep with different anthelmintics or not treated. These values were similar to those observed in this study (Table 4).

For plasmatic albumin and total protein, significant effects between treatments were not found (Table 4). In the early period there was decrease of protein values, which could be justified by the spoliation of *H. contortus* in the acute phase, at the experiment beginning. However, after the 3rd week it was observed normal values for this variable (Table 4).

Table 4 – Average of erythrocyte, hematocrit, protein and albumin plasmatic from lambs infected with *Haemonchus contortus* and treated orally with *Piptadenia viridiflora* (283 mg kg^{-1} bw) or levamisole phosphate (0.6 mg kg^{-1} bw)

	Collection period (days)					
Treatmens	0	7	14	21	Reference[a]	CV (%)
		Erythrocyte ($\times 10^6$ μL^{-1})				
Control	11.57 a	11.00 a	10.91 a	10.11 a		
Levamisole	11.24 a	9.08 a	9.15 a	9.72 a	9-15	17.35
P. viridiflora	10.84 a	11.87 a	11.46 a	9.73 a		
* Interaction period	11.22 A	10.64 A	10.63 A	9.85 A		
		Hematocrit (%)				
Control	34.67 Aa	31.75 Aa	34.97 Aa	30.35 Aa		
Levamisole	34.00 Aa	29.35 Aa	30.85 Aa	29.85 Aa	27-45	18.11
P. viridiflora	33.22 Aa	35.35 Aa	35.12 Aa	35.17 Aa		
		Total protein (%)				
Control	5.73 a	7.29 a	7.91 a	6.71 a		
Levamisole	5.70 a	6.76 a	7.35 a	5.66 a	6-7.9	16.25
P. viridiflora	5.67 a	5.36 a	6.44 a	6.12 a		
* Interaction period	5.70 B	6.47 B	7.23 A	6.17 B		
		Albumin (g dL^{-1})				
Control	4.02 a	3.60 a	4.10 a	4.17 a		
Levamisole	2.87 a	3.42 a	2.87 a	4.27 a	2.4-3	26.68
P. viridiflora	3.27 a	3.42 a	5.30 a	3.52 a		
* Interaction period	3.39 B	3.48 B	3.99 B	4.09 B		

Different letters in uppercase and lowercase line in columns differ significantly by the Scott-Knott test ($P < 0.05$). [a] Reference range for sheep (Sharp, Corp., 2015)

The plasmatic albumin values can be indicator of protein deficit or chronic lesions in the live (Reis et al. 2007). The albumin values found in this study were within the normal range and probably did not occurred protein deficit, despite infection with this nematode. Possibly adequate diet administrated to the animals might has contributing to the maintenance of plasma albumin. In another study in sheep treated with albendazole, ivermectin and levamisole, closantel, moxidectin was also reported similar concentrations of serum albumin between treated or not treated groups (Holsback et al. 2013).

The results indicated that aqueous extract of *P. viridiflora* administered at 283 mg (dm) kg-1 bw during three consecutive days did not interfere with normal standards showing no toxicity to blood parameters evaluated.

4 Conclusion

Pipitadenia viridiflora shows promising potential as alternative treatment of haemonchosis. The low concentrations of leaf extracts show high efficacy to EHI and LDI. Aqueous extract administered at 283 mg (dm) kg^{-1} bw during three consecutive days promotes moderated efficacy to FEC reduction and no clinical or blood disorders. Futures analyses with higher doses, other extraction processes and higher administration frequency could promote better *in vivo* efficacy to alternative control of *Haemonchus contortus*.

References

Adamu, M, Naidoo, V, Eloff, JN (2013) Efficacy and toxicity of thirteen plant leaf acetone extracts used in ethnoveterinary medicine in South Africa on egg hatching and larval development of *Haemonchus contortus*. BMC Vet Res, 9:38.

Adiele, RC, Fakae, B, Isuzu, IU (2013) Anthelmintic activity of *Securidaca longepedunculata* (Family: Polygalaceae) root extract in mice, *in vitro* and *in vivo*. Asian Pac J Trop Med 841-846.

Araújo, RN, Lima, S (2005) Infecções helmínticas em um rebanho leiteiro na região Campo das Vertentes de Minas Gerais. Arq Bras Med Vet Zootec 57:186-193.

Athanasiadou, S, Kyriazakis, I (2004) Plant secondary metabolites: antiparasitic effects and their role in ruminant production systems. Proc Nutr Soci 63:631-639.

Athanasiadou, S.; Githiori, J.; Kyriazakis, I. Medicinal plants for helminth parasite control: facts and fiction. Animal, v.1, p.1392-1400, 2007.

Bezerra, JCB, Silva, IA, Ferreira, HD, Ferri, PH, Ferri, SC (2002) Molluscicidal activity against *Biomphalaria glabrata* of Brazilian Cerrado medicinal plants. Fitoterapia 73:428-430

Bizimenyera, ES, Githiori, JB, Eloff, JN, Swan, GE (2006) *In vitro* activity of *Peltophorum africanum* Sond. (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *Trichostrongylus colubriformis*. Vet Parasitol 142:336-343.

Borges, CCL (2003) Atividade *in vitro* de antihelmínticos sobre larvas infectantes de nematódeos gastrintestinais de caprinos, utilizando a técnica de coprocultura quantitativa (Ueno, 1995). Parasitol Latinoam 58:142 -147.

Bräunlich, M, Slimestad, R, Wangensteen, H, Brede, C, Malterud, KE, Barsett, H (2013) Extracts, Anthocyanins and Procyanidins from *Aronia melanocarpa* as Radical Scavengers and Enzyme Inhibitors. Nutrients 5:663-678.

Bukowska B, Kowalska S (2004) Phenol and catechol induce prehemolytic and hemolytic changes in human erythrocytes. Toxicol Lett 152: 73-84.

Calderón-Quintal, JA, Torres-Acosta, JFJ, Sandoval-Castro, CA, Alonso, MA, Hoste, H, Aguilar-Caballero, A (2010). Adaptation of Haemonchus contortusto condensed tannins: can it be possible? Arch Med Vet 42:165-171.

Camurça-Vasconcelos, ALF, Bevilaqua, CML, Morais, SM., Vieira, LS., Maciel, MV, Braga RR., Macedo, ITF (2008) Anthelmintic activity of *Lippia sidoides* essential oil on sheep gastrointestinal nematodes. Vet Parasitol 154:167-170.

Coles, GC, Bauer, C, Borgsteede, FH, Geerts, S, Klei, TR, Taylor, MA, Waller, PJ (1992) World Association for the Advancement of Veterinary Parasitology

(WAAVP) – methods for detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol 44:35–44.

Cooper, KM, Whelan, M, Danaher, M, Kennedy, DG (2011) Stability during cooking of anthelmintic veterinary drug residues in beef. Food Addit Contamt 28: 155–165.

Costa, FM. Influência da estrutura da vegetação na seleção da dieta de ovinos em pastejo, em área de cerrado. Montes Claros, 2010. 78p. Dissertação (Mestrado). Universidade Federal de Minas Gerais/Instituto de Ciências Agrárias, 2010.

Cringoli, G., Veneziano, V., Rinaldi, L., Sauve, C., Rubino, R., Fedele, V.,Cabaret, J. 2007. Resistance of trichostrongyles to benzimidazoles in Italy: a first report in a goat farm with multiple and repeated introductions. Parasitol Res 101:577–581.

Duarte, ER, Silva, RB, Vasconcelos, VO, Nogueira, FA, Oliveira, NJF (2012) Diagnóstico do controle e perfil de sensibilidade de nematóides de ovinos ao albendazol e ao levamisol no norte de Minas Gerais. Pesq Vet Bras 32:147-152.

Eguale, T, Tilahun, G, Debella, A, Feleke, A, Makonnen, E (2007) *In vitro* and *in vivo* anthelmintic activity of crude extracts of *Coriandrum sativum* against *Haemonchus contortus.* J Ethnopharmacol 110:428–33.

Ferreira, LEA, Castro, PMN, Chagas, ACS. França, BSC., Beleboni, ARO (2013) *In vitro* anthelmintic activity of aqueous leaf extract of *Annona muricata* L.

(Annonaceae) against *Haemonchus contortus* from sheep. Exp Parasitol 134:327-332.

Gandhi VM, Cherian KM (2000) Red cell haemolysis test as an in vitro approach for the assessment of toxicity of karanja oil. Toxicol in Vitro 14:513-516.

Githiori, JB, Höglund, J, Waller, PJ, Baker, RL (2003) The anthelmintic efficacy of the plant, *Albizia anthelmintica*, against the nematode parasites *H. contortus* of sheep and *Heligmosomoides polygyrus* of mice. Vet Parasitol 116:23–34.

Githiori JB, Athanasiadou S, Thamsborg SM (2006) Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. Vet Parasitol 139:308- 320.

Gordon, HM, Whitlock, HV (1939) A new technique for counting nematode eggs in sheep faeces. J Coun Sci Resc Aust 12: 50-2.

Goswami, S, Pandey, A, Tripathi, P, Singh, A, Rai, A (2011) An *in vitro* evaluation of the anthelmintic activity of *Hedychium spichatum* rhizomes and *Zingiber zerumbet* rhizomes on the Pheritima Posthuma model: A comparative study. Pharmacogn Res 3:140-142.

Hiermann, A, Kartnig, TH, Azzam, S (1986) Ein Beitrag zur quantitativen Bestimmung der Procyanidine in Crataegus. Sci Pharm 54:331-337.

Hoste, H, Jackson, F, Athanasiadou, S; Thamsborg, SM, Hoskin, SO (2006) The effects of tannin rich plants on parasitic nematodes in ruminants. Trends Parasitol 22:253-261.

Iqbal, Z, Lateef, M, Jabbar, A, Mohammad, R, Khan, MN (2005) Anthelmintic activity of *Calotropis procera* (Ait) Ait F. flowers in sheep. J Ethnopharmacol 102:256–261.

Jabbar, A, Zaman, MA, Iqbal, Z, Yaseen, M, Shamim, A (2007) Anthelmintic activity of *Chenopodium album* (L.) and *Caesalpinia crista* (L.) against trichostrongylid nematodes of sheep. J Ethnopharmacol 114:86-91.

Kakar, SA, Tareen RB, Kakar MA, Jabeen H, Kakar SR, Al-Kahraman Y.M.S.A., Shafee M (2013) Screening of antibacterial activity of four medicinal plants of Balochistan-Pakistan. Pakistan J Bot 44:245-250.

Kerboeuf, D, Riou, M, Guégnard, F (2008) Flavonoids and related compounds in parasitic disease control. Mini Rev Med Chem 8:116-128.

Krychak-Furtado, S. Alternativas fitoterápicas para o controle da verminose ovina no estado do paraná: testes *in vitro* e *in vivo*. 2006. 147 f. Tese (Doutorado em Agronomia) - Departamento de fitotecnia e fitossanitarismo. Universidade Federal do Paraná, Curitiba, 2006.

Lange, KC, Olcott, DD, Miller, JE, Mosjidis, JA, Terrill, TH, Burke, JM, Kearney, MT (2006) Effect of *Sericea lespedeza* (Lespedeza cuneata) fed as hay, on natural and experimental *Haemonchus contortus* infections in lambs. Vet Parasitol 141:273–278.

Leathwick, DM, Pomroy, WE, Heath, AC, 2001. Anthelmintic resistance in New Zealand. NZ Vet J 49:227–235.

Longo, F, Rakotonirinab, S, Rakotonirinac, A, Savinea, J (2008) *in vivo* and *in vitro* effects of *Bidens pilosa* L. (asteraceae) leaf aqueous and ethanol extracts on primed-oestrogenized rat uterine muscle. Afr J Trad Cam 5:79 – 91

Lorenzi, H, Matos, FJA (2002). Plantas medicinais no Brasil: nativas e exóticas cultivadas. Nova Odessa: Instituto Plantarum, 2002. v. 2.

Lorenzi, H (2009) Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil. vol. 2. 3ª ed. 2009. 384p.

Marley, CL, Cook, R, Barrett, J, Keatinge, R, Lampkin, N H (2003) The effect of birdsfoot trefoil (Lotus corniculatus) and chicory (Cichoriumintybus) on parasite intensities and performance of lambsnaturally infected with helminth parasites. Vet Parasitol 112:147-155.

Nery, PS, Nogueira, FA, Oliveira, NJF, Martins ER, Duarte, ER (2012) Efficacy of extracts of immature mango on ovine gastrointestinal nematodes. Parasitol Res 111:2467-2471.

Nery, PS, Nogueira, FA, Martins, ER, Duarte, ER (2010) Effect of *Anacardium humile* on the larval development of gastrintestinal nematodes of sheep. Vet Parasitol 171:361-364.

Nogueira, FA, Fonseca, LD, Silva, RB, Ferreira, AVP, Nery, PS, Geraseev, LC, Duarte, ER (2012) *In vitro* and *in vivo* efficacy of aqueous extract of *Caryocar brasiliense* Camb. to control gastrointestinal nematodes in sheep. Parasitol Res 111:325-330.

Paula-Júnior, W, Rocha, FH, Donatti, L, Fadel-Picheth, CMT, Weffort-Santos, AM (2006) Leishmanicidal, antibacterial, and antioxidant activities of *Caryocar brasiliense* Cambess leaves hydroethanolic extract. Braz J Pharmacogn 16:625-630.

Peneluc, T.; Domingues, L.F.; Almeida, G.N.; Ayres, M.C.C.; Moreira, E.L.T.; Cruz, A.C.F.; Bittencourt, T.C.B.S.C.; Almeida, M.A.O.; Batatinha, M.J.M. Atividade antihelmíntica do extrato aquoso das folhas de Zanthoxylum rhoifolium Lam. (Rutaceae). Revista Brasileira Parasitologia Veterinária, v.18, p.43-48, 2009.

Pequeno, NF, Soto-Blanco, B (2006) Toxicidade *in vitro* de plantas tóxicas: avaliação do teste de ação hemolítica. Acta Sci Vet 34: 45-48.

Power, C, Danaher, M, Sayers, R, O'Brien, B, Whelan, M, Furey, A, Jordan, K (2013) Investigation of the persistence of rafoxanide residues in bovine milk and fate during processing. Food Addit Contam 30:1087-1095.

Qadir, S, Dixit, AK, Dixit, P (2010) Use of medicinal plants to control *Haemonchus contortus* infection in small ruminants. Vet World 11:515-518.

Saeg. 2007. Sistema para Análises Estatísticas, Versão 9.1. Viçosa: Fundação Arthur Bernardes – UFV.

Schnyder, M, Torgerson, PR, Schönmann, M, Kohler, L, Hertzberg, H (2005) Multiple anthelmintic resistance in *Haemonchus contortus* isolated from South African Boer goats in Switzerland. Vet Parasitol 128:285-290.

Soro, D, Koné, WM, Bonfoh, B, Dro, B, Toily, KB, Kamanzi, K (2013) *In vivo* anthelmintic activity of *Anogeissus leiocarpus* Guill & Perr (Combretaceae) against nematodes in naturally infected sheep. Parasitol Res 112:2681-1288.

Tokarnia, CH, Peixoto, PV, Brito, MF, Duarte, MD, Brust LAC (1999) Estudos experimentais em bovinos com plantas cianogênicas. Pesq Vet Bras 19:84-90.

Vetter, J (2000) Plant cyanogenic glycosides. Toxicon 38:11-36.

CAPTER 4

Ximenia americana L. (Olacaceae), selected from Cerrado to control of *Haemonchus contortus* in lambs

Abstract

Resistance to anthelminthics has been common in different continents. *Ximenia Americana* is an plant from Cerrado selected naturally by sheep and could be source of bioactive compounds for development of new farmacs. In this study, *in vitro* and *in vivo* efficacy this vegetal was evaluated for lambs naturally infected with *Haemonchus contortus*. The presence of tannins and flavonoids for aqueous and ethanolic extracts of the leaves were indicated by HPLC-DAD. These extracts at 0.6 and 1.2 mg mL^{-1}, respectively, showed 100% efficacy to egg hatch inhibition (EHI). In larval development inhibition (LDI) test with quantitative cultures, crude powder ≥ 333.3 mg g^{-1} or aqueous extract ≥ 22.70 mg mL^{-1} promoted L3 mean lower than those observed for the control with water ($P<0.05$) and the estimated LC90 were 41.96 and 8.1 mg kg^{-1} bw of feces respectively. Maximum tolerated dose for male and female mice was > 22.70 mg kg^{-1} bw in probe gavage via. *In vivo* of lambs crude powder and aqueous extract were orally administered at 157.35 and 30 mg (dm) kg^{-1} bw respectively during three consecutive days. There was no efficacy. Low concentrations of extracts promoted high efficacy for EHI and LDI. The oral treatment with crude powder and aqueous extract is not promising *in vivo* anthelminthic efficacies not interference with normal clinical standards.

Keywords: Brasilian savanna, sheep, antihelminthic plant, phytochemical composition, toxicity.

1 Introducion

Ximenia americana L. (Olacaceae) it is popularly known as “ameixa-do-cerrado” (Lorenzi, 2009) is is found frequentily in the Cerrado and Africa India, New Zeland, Central America and Sul America (Sacande; Vautier, 2006). *X. americana* is characterized as shrub 3-4 meters high, with a prickly small tree (Matos, 2007). It has healing action, which can be justified by the presence of certain substances, such as tannins (Veras; Morais, 2004).

In prelimary study of Cerrado Biome, *X. americana* was naturally selected by lambs grazing during the dry and rainy seasons, with selectivity index 2.34-16.03% (Morais-Costa, et al., 2014), when this ratio is ≥ one indicates that this vegetal species is important in the animal's diet . Then the purpose in this study was to analyze *in vitro* and *in vivo* extracts of *Ximenia americana* to control *Haemonchus contortus*.

2 Material and methods

2.1 Study area

The research was conducted in a rural area of Montes Claros of North of Minas Gerais state, Brazil (W 43º50’33.56”; S 16º41’10.05”). The climate of this region is tropical wet and dry (Aw) according to the Köppen classification and marked by long dry season from May to October and rainy period in November to April at 2013.

2.2 Production of vegetal extratcts

Healthy leaves of *Ximenaia americana* (Fig. 1) from Cerrado Biome were selected and dried to constant weight in a forced air circulating drier at 40ºC for 72 h. Dried leaves were grinded in a wiley mill and then were stored in paper bags, free of light incidence. Samples of the plant were stored in the Montes Claros Herbarium (HMCMG) of the Universidade Estadual de Montes Claros-Brazil, with voucher specime, 211.

Figure 1 - *Ximenia Americana* L. (Olacaceae)

Aqueous extract of dried leaves were held in a distilled water bath at 40°C for 60 min. Ethanolic extract was obtained by maceration of the dried leaves in absolute ethyl alcohol (PA), in glass amber containers, kept in a dark place and stored for seven days. After this extraction, filtration was held through a gauze funnel. The extracts were dehydrated at 40ºC for 48 h, until obtaining the residue with constant weight and stored in paper bags in darkness and refrigerated at ~4ºC until use (Adapted Nery et al., 2010).

2.3 High-performance liquid chromatography (HPLC) analyses and proanthocyanidin quantification

A Waters Alliance 2695 HPLC system composed of a quaternary pump, an autosampler, a photodiode array detector (DAD) 2996, and a Waters Empower Pro data handling system was used (Waters Corporation, Milford, USA). The analyses were performed on a LiChrospher 100 RP-18 column (250 × 4 mm i.d., 5 μm; Merck, Darmstadt, Germany) combined with a LiChrospher 100 RP-18 guard column (4 × 4 mm i.d., 5 μm; Merck) at 40 ºC. Water (A) and acetonitrile (B) were used as eluents, both containing 0.1% (v/v) of H_3PO_4 at a flow rate of 1.0 mL min^{-1} as follows: 0 min, 95% A and 5% B; 60 min, 5% A, 95% B, followed by 10 min of isocratic elution. Solvents used were of HPLC grade (Merck, Germany) and were degassed by sonication before use. The chromatograms were obtained at 210 nm, and the UV spectra were recorded on-line from 190 to 400 nm.

The aqueous and ethanolic extracts were dissolved in methanol (HPLC-grade), ultrapure water, or hydroethanolic solutions, according to their solubility, to concentrations of 10 mg mL^{-1}. After centrifugation at 8 400 x *g*, the sample solutions (10 μL) were automatically injected into the apparatus.

Total proanthocyanidin content of the dried aqueous extracts was determined by measuring at 540 nm the absorbance of the cyanidin chloride resulting from acid-catalyzed solvolysis with *n*-BuOH/HCl 37% (95:5), according to the method described by Hiermann et al. (1986). Each sample was analyzed in triplicate and the results expressed as mean ± standard deviation. The total proanthocyanidin content, expressed as cyanidin chloride, was calculated using the following formula:

$$\% = \frac{A_{\text{sample}} - A_{\text{blank}} \times 4.115}{m_{\text{sample}}}$$

A is the measured absorbance at 420 nm, and m_{sample} is the sample weight in g.

2.4 Egg hatching inhibition (EHI)

The aqueous and ethanolic extract of the dried leaves was standardized at 1.2 mg mL^{-1} and diluted in sterile distilled water. These extracts were used in EHI tests immediately after dissolution as described Coles et al. (1992).

Flotation, sedimentation, and filtration techniques in saturated NaCl solution were conducted to obtain nematode eggs from feces of two Santa Inês lambs infected only with *Haemonchus contortus* and with an average fecal egg count (FEC) >1000 g^{-1}, determined using the modified McMaster technique (Gordon and Whitlock, 1939).

Experimental mixtures contained: 100 μl fecal suspension with an average of 80 fresh eggs, and 100 μl of the extract, at final concentrations 1.2-0.037 mg mL^{-1} or two positives controls with solution levamisole phosphate (0.3 mg mL^{-1}) or ivermectin (16 μ mL^{-1}) or a negative control with sterile distilled water. The samples were homogenized and incubated in a BOD incubator at 28°C for 48 h. Subsequently, 15 μl Lugol's solution was added to each tube, which were then stored at 4°C for subsequent counting of unembryonated eggs, embryonated eggs, first stage larvae (L1; Coles et al. 1992).

The number of L1 relative to the total number of eggs plus L1 was determined for each repetition and subjected to variance analysis. The means were compared using the

Tukey test at 5% significance. Probit regression was employed to determine the concentrations sufficient to inhibit 90% (lethal concentration, LC_{90}) of egg hatching using the statistical package, Saeg 9.1 (2007).

The formula of Coles et al. (1992) was used to determine the EHI effectiveness:

% effectiveness = 100 x (1 – mean of L1 / mean eggs eggs + L1)

2.5 *In vitro* larval development inhibition (LDI)

The effectiveness of the crude powder and aqueous extract were evaluated by adapted coproculture quantitative methodology (Borges, 2003; Nery et al. 2010, Nogueira, 2012) using fresh feces of lambs with *Haemonchus contortus* mono-infection**.**

Eigth treatments were performed, each with five replicates, including two positive control: 2 mL of solution 16 µg g^{-1} ivermectin (final concentration) or 2 mL of solution 0.1 mg g^{-1} levamisole phosphate (final concentration) and a negative control of 2 mL of sterile purified water, both added to 2 g feces.

The five treatments for crude powder for standardized at 83.3-333.3 mg of dw g^{-1} of fecal culture final concentration and and five treatments aqueous extract, was standardized at 1.42-22.70 mg dw g^{-1} of fecal culture. The samples were incubated in a BOD incubator at 28°C for seven days and assessed for presence of infective larvae (L3). The following formula, adapted from Borges (2003), was used to determine the percent reduction of larva g^{-1} of feces (LPGF):

% efficacy = 100 × (1 - LPGF of the treated group/LPGF of the treated group)

The data were transformed in log (x + 1) and submitted to variance analysis. The means were compared through the Duncan test at 5% probability and LC_{90} was determined by probit analysis using the statical package SAEG® 9.1 (2007).

2.6 Toxicity in mice

The mouse toxicity testing was performed as Walum (1998) to determine the maximum tolerated dose (MTD) for an adult mouse. Using probe gavage 22µL of aqueous extract of *X. americana* was added for four Balb C mice (2 males and 2 females) with average 22g of body weight and 6 to 8 weeks.

For the first and second days the extract was diluted at 100 and 10 times respectively in 1x PBS. In the third and fourth days the extract was administered at 22.70 mg ml^{-1}. The mice were euthanized by cervical dislocation on day 5 after extract administration.

2.7 *In vivo* anthielmintic test

Two experiments were perfomed crude powder or aqueous extract. For each analyses were used on 24 Santa Inês lambs with average 26.5 kg bw of both sexes, with 4-to-8-month-old. Prior to the beginning of the trial, all sheeps were administered albendazole (10 mg kg^{-1} bw) and phosphate levamisole (0.6 mg kg^{-1} bw), to ensure that were worm-free.

During 10 days of adaptation, the animals were individually confined which fed balanced diet containing sorghum silage, concentrate, mineral premix and water *ad libitum*, according to the age category requirement.

The animals, with zero fecal egg count (FEC), were infected with 800 L3 for $10kg^{-1}$ bw from lambs naturally contaminated in *H. contortus*. Twenty-eight days post-infection, sheep were assigned to one of three homogeneous groups based on FEC, weight, and sex.

One untreated animals served as the negative control, other group was orally administered phosphate levamisole (0.6 mg kg^{-1} bw) via subcutaneous and represented the positive control, the third group was administered crude powder of *X. americana*, at 157.35 mg (dm) kg^{-1} bw during three consecutive days, the third group was administered aqueous vegetal extract of *X. americana*, by esophageal gavage, at 30.375 mg (dm) kg^{-1} bw during three consecutive days and the positive control.

The mean of two counts of FEC was obtained for each collection periods. The McMaster technique was utilized with the addition of saturated NaCl for FEC in duplicate (Gordon and Whitlock, 1939).

Initial FEC value was recorded based on the mean values of three days prior to initiation of treatment. Subsequently, mean FEC was determined for three periods post-treatment to efficacy analysis: 7, 8 and 9 days (first period), 14, 15 and 16 (second period) and 21, 22 and 23 (third period). Fecal samples were cultured to obtain larvae for identification and confirmation of mono-infection by *H. contortus* (Ueno & Gonçalves, 1998).

The data relating FEC values were previously transformed into log (x + 10) and subjected to analysis of variance and the means were compared by Duncan test ($p<0.05$). A formula adapted from Coles et al. (1992) was used to determine the percentage efficacy in FEC reduction:

Efficacy = 100 x (1 – mean FEC of treated group/mean FEC of control group)

All procedures were performed in accordance with the principles of animal experiments approved in the 275/2013 protocol of the Ethics Committee on the use of animals (CEUA) of the Federal University of Minas Gerais, Brazil.

3 Results and discussion

3.1 Extracts characterization

The presence of polyphenols for both extracts was indicated by HPLC-DAD chromatograms, which clearly indicate the predominance of peaks corresponding to polar compounds, with UV spectra compatible with polyphenols (Fig. 2, 3). In addition, the HPLC chromatograms both extracts showed present major peaks with UV spectra characteristic of flavonoids (λ 255.6 and 348.3 nm aqueous and λ 255 and 353.1 to ethanolic). The quantification total de proanthocyanidin concentrations for aqueous and ethanolic extracts were 0.3% ± 0.1 and 0.4% ± 0.2 respectively.

In general, the compounds found in *X. americana* were saponins, glycosides, flavonoids, tannins, phenolics, alkaloids, quinines, and terpenoids. In addition, the plant is rich in fatty acids and glycerides, and the seeds contain cyanide derivatives (Monte et al. 2012). Tannins can exert direct antihelminthic action, interfering with the natural cycle of helminths, or indirectly, by inhibiting the degradation of ruminal protein (Ketzis et al. 2006).

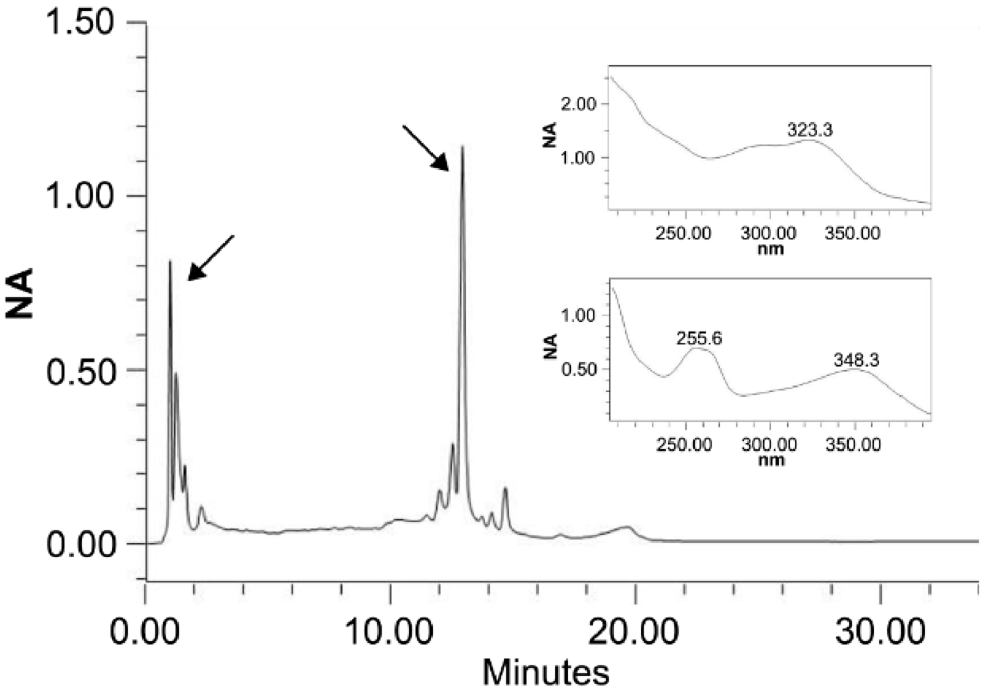


Figure 2 - HPLC-DAD chromatograms obtained for the aqueous extracts of the *Ximenia americana*

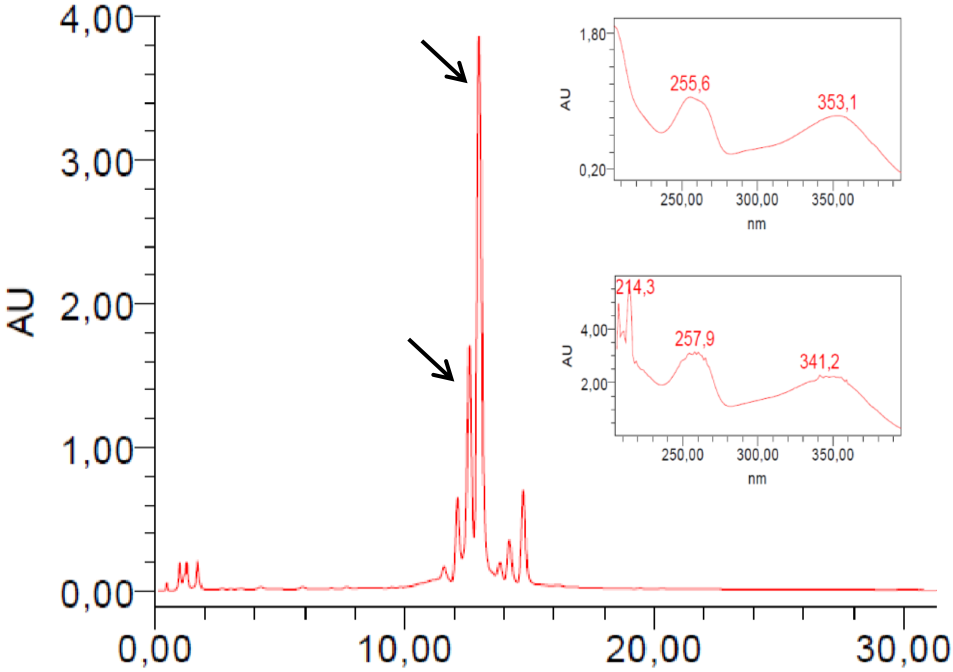


Figure 3 - HPLC-DAD chromatograms obtained for the aqueous ethanolic of the *Ximenia americana*.

3.2 Egg hatching inhibition

The effectiveness was directly related with increasing concentration of the aqueous extract of *X. americana* leaves. The aqueous and ethanolic efficacies were at 42.58-100% and 57.41-100% respectively (Table 1), and LC_{90} for these extracts were 2.17 and 2.10 mg mL^{-1} respectively. All concentrations tested for both extracts promoted significant reduction for hatched larvae mean when compared to the negative control with distilled water. The aqueous extracts showed greater inhibition of larval hatching, whereas ethanol extract showed the best inhibition of early embryonic development (Table 1).

Table 1 - Aqueous and ethanolic extracts of *Ximenia americana* leaves in egg hatching of *Haemonchus contortus*

Treatments	Unembryonated egg mean	Embryonated egg mean	L1 mean	Eggs + L1	Efficacy* (%)
Aqueous extract (mg mL^{-1})					
0.6	1.4^{b}	27.8^{a}	0.0^{e}	29.2	100.0
0.3	1.8^{b}	23.6^{a}	3.0^{e}	28.4	96.41
0.15	2.2^{b}	25.4^{a}	11.6^{d}	39.2	86.12
0.075	0.8^{b}	14.6^{b}	38.2^{c}	53.8	54.30
0.037	0.8^{b}	2.33^{c}	48.0^{b}	51.13	42.58
Variation coefficient (%)	8.12	21.15	14.61		
Ethanolic extract					
1.2	0.4^{d}	2.4^{bc}	0.0^{f}	2.8	100.0
0.6	42.6^{c}	0.0^{c}	8.2^{de}	50.8	90.19
0.3	55.0^{b}	0.0^{c}	12.0^{d}	67.0	85.65
0.15	44.8^{b}	5.4^{b}	21.4^{c}	71.6	74.40
0.075	0.0^{d}	13.2^{a}	35.6^{b}	48.8	57.41
Levamisole (0.3 mg mL^{-1})	78.0^{ab}	0.0^{a}	2.6^{b}	80.6	100.0
Ivermectin (16µ mL^{-1})	78.8^{a}	0.0^{a}	0.0^{b}	0.0	96.88
Sterile distilled water	0.0^{d}	0.0^{ab}	83.6^{a}	83.6	___
Variation coefficient (%)	9.53	72.20	15.47		

Mean followed by a different letter in the columns indicates significant differences by Tukey test ($P < 0.05$).

*% efficacy = 100 x (1 - L1/initial egg + L1).

Research with other Cerrado plants has also reported high efficacies in EHI tests. The Aqueous extract of *C. brasiliense* fruit's skin in the EHI test at 15 mg ml^{-1} presented anthelminthic efficacy of 98.7 and LC_{90} was 7.35 mg ml^{-1} (Nogueira et al. 2012). The essential oil from *Chenopodium ambrosioides* (Erva-de-Santa-Maria) administered in goats at 0.2 mg ml^{-1} bw reduced EHI of *H. contortus* but had no effect (P>0.05) on the reduction of number of eggs or adult nematodes (Ketzis et al. 2002). Aqueous extract of *A. muricata*, cerrado specie inhibited the hatching of *H. contortus* eggs by 84.91% with for dilution 50%.

3.3 Larval development inhibition

The crude powder 333.3 mg g^{-1} showed mean of L3 significantly lower than those observed for the control with water (P<0.05). There was increase to effectiveness of LDI with increased concentration (Table 2) and estimated LC_{90} was 41.96 mg g^{-1} of feces.

Table 2 - Mean *Haemonchus contortus* larvae per gram of feces (LPGF) in quantitative cultures treated whit different concentrations of the crude powder of the *Ximenia americana* leaves

Tratamentos (mg g^{-1})	LPG*	Eficácia (%)
333,3	0,0 ^{c}	100
250,0	6,6 ^{c}	99,1
166,7	6,6 ^{bc}	99,1
83,3	26,5 ^{b}	96,3
Levamisol (0.1 mg g^{-1})	0,0 ^{c}	100.0
Ivermectin (16 µ mL^{-1})	0 ^{c}	100.0
Sterile distilled water	713,3 ^{a}	---

Means followed by different letters in the columns indicate significant differences (P< 0.05), by Duncan test.
Coefficient of variation LDI: 20.10%
*LPGF: number of larvae (L3) per gram of feces
Efficacy: % efficacy = 100 x (1 - LPGF of the treated group/LPGF of the treated group).

The aqueous extract 22.70 mg mL^{-1} showed mean of L3 significantly lower than those observed for the control with water (P<0.05). There was increase to effectiveness of LDI with increased concentration (Table 3) and estimated LC_{90} was 8.1 mg g^{-1} of feces.

Table 3 - Mean *Haemonchus contortus* larvae per gram of feces (LDPG) in quantitative cultures treated whit different concentrations of the aqueous extract of the *Ximenia americana* leaves

Treatments	LPGF *	Efficacy (%)
Aqueous extract (mg g^{-1})		
22.70	0 [e]	100
11.34	25 [d]	96.88
5.67	165 [c]	79.38
2.84	190 [c]	76.25
1.42	275 [d]	65.63
Levamisole phosphate (0.1 mg g^{-1})	0 [f]	100.00
Ivermectin (16µ mL^{-1})	0 [f]	100.00
Sterile distilled water	800 [a]	---

Means followed by different letters in the columns indicate significant differences (P< 0.05), by Duncan test.
Coefficient of variation LDI: 3.04%
*LPGF: number of larvae (L3) per gram of feces
Efficacy: % efficacy = 100 x (1 - LPGF of the treated group/LPGF of the treated group).

The aqueous extract from *Caryocar brasiliense* fruit peels, popularly named as "pequi", assayed at 200 mg mL^{-1} significantly inhibited the development of *H. contortus* larvae from sheep with efficacy of 94.8%. Ferreira et al. (2013), demonstrated that aqueous extract of *Annona muricata* (Panã) leaves at 50% concentration inhibited larval hatching in 84.9%. Nery et al. (2010), evaluated extracts of leaves *Anacardium humile* A. St.-Hil. (Anacardiaceae), against different species of gastrointestinal nematodes in sheep.

3.4 Toxicity test

For evaluated concentrations, no clinical signs of toxicity for mucosal tissues or changes in animal behavior or deaths during were observed in the four days of extract administration. During the autopsy, macroscopical alterations were no observed in the liver, kidneys, spleen, lungs or other viscera. Then maximum tolerated dose for male and female mice was > 22.70 mg kg^{-1} bw.

3.5 *In vivo* anthelmintic activity

There was no efficacy for crude powder or aqueous extract. This may be due because significant physiological differences are expected in conditions in vitro and in vivo (Githiori et al. 2006). Eguale et al. (2007), reported that the lower effect *in vivo* can be attributed the biotransformation of some of the active components inside the digestive tract of the host.

Conclusion

Ximenia americana shows promising potential as alternative treatment of haemonchosis. The low concentrations of leaf extracts show high efficacy to EHI and LDI. Crude powder administered at 157 mg (dm) kg^{-1} bw or aqueous extract at 30.375 mg (dm) kg^{-1} bw during three consecutive days dont promoted efficacy to FEC reduction. Futures analyses with higher doses, other extraction processes and higher administration frequency could promote better in vivo efficacy to alternative control of *Haemonchus contortus*.

References

Borges, CCL (2003) Atividade *in vitro* de antihelmínticos sobre larvas infectantes de nematódeos gastrintestinais de caprinos, utilizando a técnica de coprocultura quantitativa (Ueno, 1995). Parasitol Latinoam 58:142 -147.

Coles, GC, Bauer, C, Borgsteede, FH, Geerts, S, Klei, TR, Taylor, MA, Waller, PJ (1992) World Association for the Advancement of Veterinary Parasitology (WAAVP) – methods for detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol 44:35–44.

Eguale, T, Tilahun, G, Debella, A, Feleke, A, Makonnen, E (2007) *In vitro* and *in vivo* anthelmintic activity of crude extracts of *Coriandrum sativum* against *Haemonchus contortus.* J Ethnopharmacol 110:428–33.

Ferreira, LEA; Castro, PMN; Chagas, ACS; França, BSC; Beleboni, ARO. In vitro anthelmintic activity of aqueous leaf extract of *Annona muricata* L. (Annonaceae) against *Haemonchus contortus* from sheep. *Experimental Parasitology*, v. 134, 327-332. 2013.

Githiori, JB, Höglund, J, Waller, PJ, Baker, RL (2003) The anthelmintic efficacy of the plant, *Albizia anthelmintica*, against the nematode parasites *H. contortus* of sheep and *Heligmosomoides polygyrus* of mice. Vet Parasitol 116:23–34.

Gordon, HM, Whitlock, HV (1939) A new technique for counting nematode eggs in sheep faeces. J Coun Sci Resc Aust 12: 50-2.

Hiermann, A., Kartnig, T.H., Azzam, S. 1986. Ein Beitrag zur quantitativen Bestimmung der Procyanidine in *Crataegus*. Scientia Pharmaceutica. 54, 331–337.

Ketzis, JK. et al. Evaluation of efficacy expectations for novel and non-chemical helminth control strategies in ruminants. *Veterinary Parasitology*, v.139, 321-335, 2006.

Lorenzi H. 2009. Árvores Brasileiras. Terceira Ed. Nova Odessa: Plantarum, Nova Odessa, SP, BR, 384 p.

Matos, F.J.A. Plantas medicinais: guia de seleção e emprego das plantas usadas em fitoterapia no Nordeste do Brasil. Fortaleza: Imprensa Universitária, 2007. p. 122-124.

Monte, F.J., Lemos, T.L.G., Araújo, M.R.S., Gomes, E.S. 2012. *Ximenia americana*: Chemistry, Pharmacology and Biological Properties, a Review. In: Phytochemicals – A Global Perspective of Their Rolein Nutrition and Health, Venketeshwer Rao, Rijeka, HRV, pp. 429–450.

Morais-Costa, F. Bastos, G. A., Soares,A. C. M., Nunes,Y R. F., Gerassev, L. C. Influência da estrutura da vegetação na seleção da dieta de ovinos em área de cerrado. Revista Caatinga. 2015. *In press*.

Nery, PS; Nogueira FA; Martins, ER, Duarte, ER. Effects of *Anacardium humile* leaf extracts on the development of gastrointestinal nematode larvae of sheep. *Veterinary Parasitology*, v.171, 361-364, 2010.

Nery, PS; Nogueira FA; Martins, ER, Duarte, ER. Effects of *Anacardium humile* leaf extracts on the development of gastrointestinal nematode larvae of sheep. *Veterinary Parasitology*, v.171, 361-364, 2010.

Nogueira, FA; Fonseca, LD; Silva, RB; Ferreira, AVP; Nery, PS; Geraseev, LC; DuartE, ER. In vitro and in vivo efficacy of aqueous extract of *Caryocar brasiliense* Camb. to control gastrointestinal nematodes in sheep *Parasitol Res*, p. 111, 325-330, 2012.

SACANDE, M.; VAUTIER , H. Ximenia americana L. Forest & Landse Denm. (112): 1-2, 2006.

Saeg. 2007. Sistema para Análises Estatísticas, Versão 9.1. Viçosa: Fundação Arthur Bernardes – UFV.

Ueno, H; Gonçalves, PC. *Manual para diagnóstico das helmintoses de ruminantes*. Tokyo. Japan International Cooperation Agency, Tokyo, 1998, p. 143.

Veras, A.O.M.; Morais, S.M. Análise dos Constituintes químicos de Ximenia americana Linn. 2004. In IX Semana Universitária e XIII Encontro de Iniciação Científica da Universidade Estadual do Ceará.

Conclusões finais

1) As principais espécies vegetais selecionadas por ovinos da Raça Santa Inês em pastejo em área de Cerrado foram: *Casearia silvestres, E. deciduum, Evolvulus* sp., *H. byrsonimifolia*, *Paullinea* sp. *Piptadenia viridiflora, Schinopsis brasiliensis e Ximenia americana.* A ação de extrato de folhas desidratadas dessas espécies foi testada *in vitro* a fim de se observar o potencial antihelmíntico frente *Haemonchus contortus*.

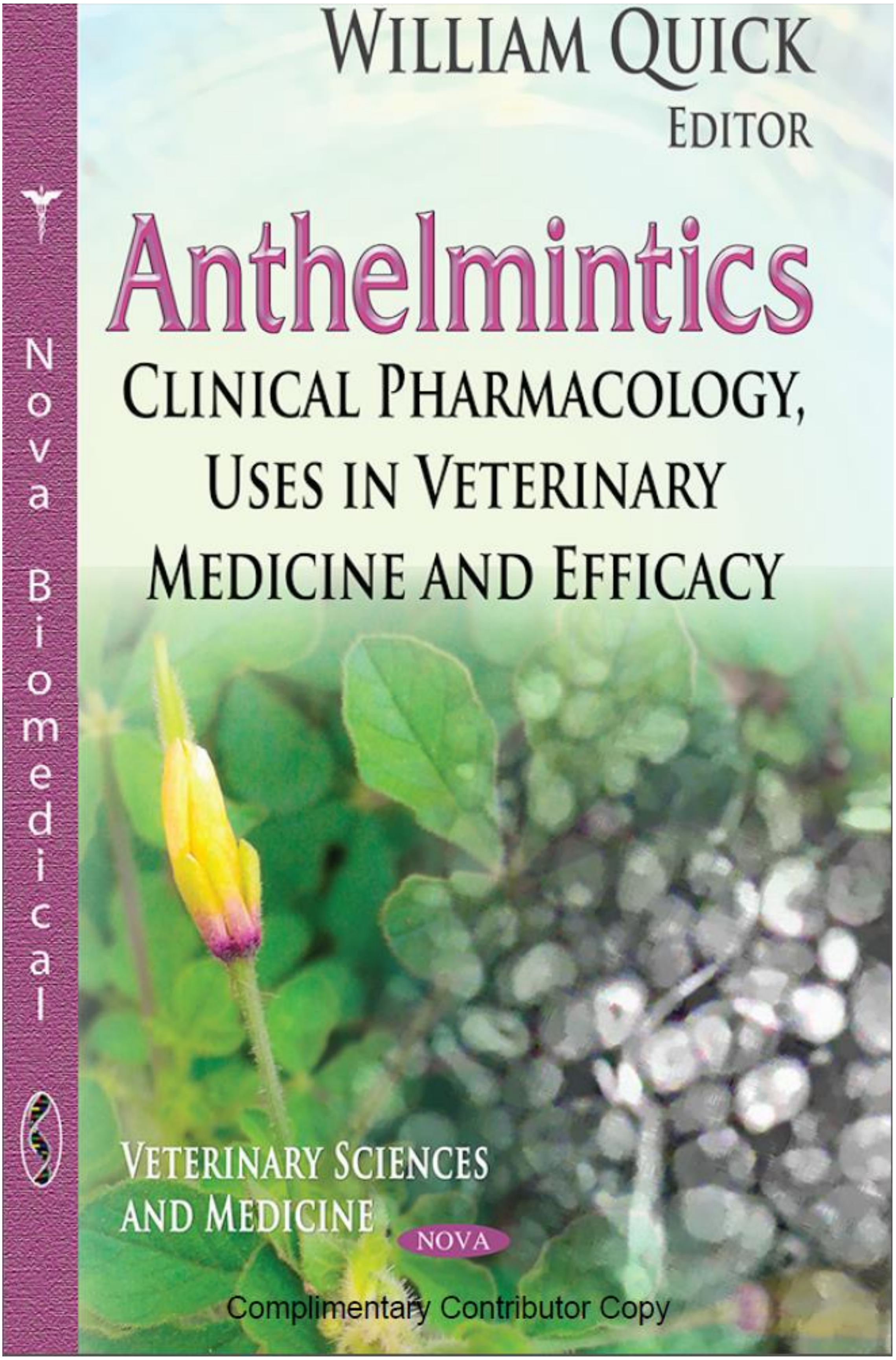
2) *Piptadenia viridiflora* e *Ximenia americana* apresentam respectivamente, baixos teores de taninos condensados (0,2 e 0,3%), embora, apresentem potencial antihelmíntico alternativo promissor no tratamento de hemoncose. Já que extratos brutos das folhas de tais plantas são eficientes em testes *in vitro* contra larvas infectantes de *Haemonchus contortus* de 86.6 a 99.6%.

3) Baixas concentrações dos extratos aquoso e etanólico das folhas de *P. viridiflora* e *X. americana* mostram elevada eficácia em testes de eclodibilidade de ovos e larvas de *Haemonchus contortus*.

4) O extrato aquoso de *P. viridiflora* administrado *in vivo* à concentração de 283 mg (ms) kg^{-1} de peso corporal durante três dias consecutivos promove moderada eficácia para a redução da ovos nas fezes sem promover sintomas de toxicidade e alterações nos padrões fisiológicos do sangue de ovinos da Raça Santa Inês experimentalmente infectados por *Haemonchus contortus*.

5) As folhas desidratas de *X. americana* administrada em 157 mg (ms) kg^{-1} de peso corporal ou extrato aquoso em 30,375 mg (ms) kg^{-1} de peso corporal durante três dias

consecutivos não promovem reduções das contagens de ovos nas fezes de ovinos da Raça Santa Inês experimentalmente infectados por *Haemonchus contortus*.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Contents

Chapter

Plants from Cerrado for the Control of Gastrointestinal Nematodes of Ruminants

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Abstract

The gastrointestinal helminthes are major limiting factors for the sheep and goat production in the world and the health of livestock depends of effective control of nematodes. The constant administration and inadequate doses of chemical anthelmintics favors the selection of resistant populations and residues these products contribute to the contamination of animal products and of the ambient. The use of herbal treatment in veterinary medicine is a promising field of research. Studies in this area require the insertion into an agroecological context, with the limiting factor to the sustainable management of natural resources involved. The phytotherapy for the parasite control is an alternative that can reduce the cost with the purchase of anthelmintics as well, preventing the emergence of anthelmintic resistance and residues in animal products. Plant species that have tannins in its constitution are known to possess anthelmintic activity, requiring, however, that their efficacies are scientifically proven. The Cerrado is an import biome with high diversity of plants rich in tannins and other metabolic with potential anthelmintic effect. This study presents a review of research on plant species, tested in the Cerrado for the control of helminths in ruminants.

Keywords: anthelmintic, nematodes, medicinal plants, Cerrado, ruminants

Introduction

The main problem in the small ruminants and limiting of economic exploitation is the gastrointestinal parasites. *Haemonchus contortus* is a nematode of abomasum and feeds of blood throughout, with high prevalence and high pathogenicity (Strong, 1993). Sheep with haemoncoses may show anemia and submandibular edema, with high mortality in young lambs and females in peripartum. Both sexes at all age levels may be intensely affected, reducing weight gain and reproductive capacity, as well as milk, wool, and hide production (Bizimenyera et al., 2006).

The treatment with anthelmintics has been intensely used to control by breeders. The constant administration and the inadequate dosages can favor the selection of the parasite populations resistant to the anthelmintics and contributes to the contamination of animal products with residues of these products (Amarante et al., 1992).

The main anthelmintics were developed during the 60's and are actually essential to control of nematodes. There are currently only three groups of broad spectrum anthelmintics and two groups of small spectrum used to control these parasites (Amarante et al., 1992). Early studies reported resistant helminthes to the group of benzimidazole and levamisoles. With the discovery of a chemical group distinct anthelmintic, avermectins, was represented an alternative treatment with a potent drug for the nematode control in domestic animals (Gopal et al., 1999). Multi-resistant nematodes have been found on several ruminant herds (Molento and Prichard 2001; Taylor et al., 2009; Wolstenholme et al., 2004; Thomaz-Soccol et al., 2004) . The possibility of anthelminthic residues in the environment and in animals reared for consumption (Hammond et al., 1997), as well as the spread of multi-resistant strains demands research into alternatives for gastrointestinal nematodes (GIN) control.

The utilization of plants containing secondary compounds such as condensed tannins may expand the organic alternatives to controlling GINs (Athanasiadou et al., 2007; Kahn and Diaz-Hernandez 2000). Phytotherapy in the control of parasitism is an alternative that can reduce the cost with the purchase of anthelmintics, and prevent the emergence of anthelmintic resistance and the presence of residues in animal products. Many plants are traditionally known as having anthelmintic activity, requiring, however, that their efficacy be scientifically proven (Vieira, 2003). Scientific validation of the anthelminthic effects and possible side-effects of plant products is necessary prior to their adoption as novel methods for control (Githiori et al., 2006).

The Cerrado biome, which covers 5% of the world flora, is the second largest source of biodiversity in Brazil (Sano, 2008). However, much of the native vegetation has been destroyed and many species are threatened of extinction, which would enable a wide use and maintenance of food, medicinal, ornamental, linseed and tannin production.

However, few studies have evaluated the anthelmintic effect of the plant species of the Cerrado for the control of GNI. Therefore, the analysis of potential plant species of this biome for helminthes control for ruminants may represent a promising strategy for the biotechnology industry and consequently for the breeders of these animals.

The Cerrado

Among the vegetation types that cover the American continent, the Cerrado presents a natural grandeur of plant species, which demonstrates the importance of education for the conservation and management of this biome. The original vegetation of the Cerrado has already been reduced by over 37 % (Felfili et al., 2002), prejudicing much of its biodiversity. Mittermeier et al. (1999) estimated that 67 % of the Cerrado areas are considered “highly modified” and only 20 % are in original condition, since the changes began with the colonization process, with the introduction of cattle, associated with rudimentary agricultural practices (Zanetti, 1994).

According Eiten (1993), the Cerrado’s flora is composed of two groups of species: thick stem trees and bushes with an undergrowth layer, consisting in a large mosaic, which includes a forest canopy formation more or less closed, containing trees with heights of 12 m tall or more. It has a woodland category, usually around six or seven meters and undergrowth stratum more or less continuous.

The herbaceous and shrub form a thick layer, especially grasses, making it difficult to distinguish individuals in both the layers as woodlands or as herbaceous due to many overhead structures being in accordance with shoots from the same root (Felfili et al., 2002).

The Cerrado *sensu strictu* is characterized by the presence of bent and twisted low trees; the shrub and herbaceous strata exhibits rapid growth during the rainy season (Ribeiro and Walter, 2008). These authors report that the Cerrado species have bent, twisted and gnarled timber trunks, leathery and rigid leaves, as adaptations to the dry environmental conditions. The most common species are represented by the Vochysiaceae and Fabaceae families, as well as species of Malpighiaceae, Anacardiaceae, Salicaceae, Rubiaceae (Felfili et al., 2002, Miranda et al., 2006), among others such as those represented by Caryocaraceae and Annonaceae families (Sales et al., 2009a; Sales et al., 2009b).

In regards climate, the average temperatures in the Cerrado areas vary between 22 º C and 27 º C (Klink and Machado, 2005) , with average annual rainfall of 1,500 mm , water deficiency ranging from three to seven months of the year, depending on the region’s seasonal (Nimer, 1989). The Cerrado’s vegetation occurs predominantly in deep and well drained soil (Reatto et al., 1998), which present a lack of nutrients such as phosphorus and nitrogen , the pH being between 4.5 and 5.5, with high aluminum frequency rates (Ribeiro and Walter, 2008).

Anthelmintic Efficacy of Plant Species from Cerrado for Control of Gastrointestinal Nematodes

In recent years, society has prioritized environmental aspects, directing ample research towards the discovery of new bioactive substances that may be used in integrated pest management, with fewer negative effects on the environment (Castro, 1989).

In an attempt to contribute with an effective alternative control of gastrointestinal nematodes in small ruminants, several researchers have attempted to test plants used in folk medicine, evaluating the efficacy and safety of the same. Plant species rich in tannins called secondary metabolites have been extensively studied. The tannins anthelmintic action may act directly by interfering with the natural cycle of helminths, or indirectly, to protect the protein intake of ruminal degradation (with increased of protein availability in the lower gastrointestinal tract), which complicates the determination of its actual antiparasitic effect (Ketzis et al., 2006).

Furthermore, the results of *in vivo* tests conducted with these forages can be influenced by natural variations in the composition of the plant (by environmental factors or of their own cycle) that alter the concentration of the tannin intake by the animals (Athanasiadou and Kyriazakis, 2004).

The anthelmintic activity, attributed to tannins is present in plant species (Hoste et al., 2006). Calderon-Quintal et al. (2010) suggest that different strains of *H. contortus* show different sensitivities to the extracts rich in tannin and further studies are needed to confirm the *in vivo* results.

Chenopodium ambrosioides L. (Chenopodiaceae) “erva-de-santa-maria”, popularly known for its anthelmintic efficacy is a plant of the Chenopodiaceae family, with stem one meter tall with leaves shaped like spears with sinuous edges. The flowers are greenish, clustered in a small bouquet. From the leaves and flowers of this plant may be extracted an essential oil consisting of a mixture of mainly ascaridiol, silvestreno and safrole, and p-cymene and isohametina. The essential oil contains 60-80 % of ascaridiol with proven anthelmintic potential, abundant in the fruit, followed by flowers and leaves (Oliver-Bever, 1983).

Ketzis et al. (2002), working with essential oil of *C. ambrosioides* (0.2 mL/Kg-1 of body weight) achieved similar thiabendazole efficacy, promoting the impracticability of all the hatched larvae of *Haemonchus contortus* in sheep. However, Vieira (1992) noted no effect when administered infused orally to cattle.

The Annonaceae family includes about 50 genera and the genus *Annona* being one of the most important. The Annonaceae is characterized mainly by presenting a class of acetogenin substances. These substances are derived from long chain fatty acids, which act as potent inhibitor of mitochondrial respiration (Wang et al., 2002). The biological activities of *Annona* extracts have been attributed to the occurrence of annonaceous acetogenins, a class of natural compounds extracted from leaves (Geum-Soog et al., 1998; Wu et al., 1995) and seeds (Chang and Wu, 2001).

Annona squamosa L. (Annonaceae), known as the Earl fruit, “pinha” or “ata” are trees that can reach up to 5 m in height with long, thin and oval leaves. Its flowers have a greenish yellow color, adapting well to climates with little rain and with a well-defined dry season (Morton, 1987). Amorim et al. (1996) evaluated the aqueous extract from *A. squamosa* leaves, *in vitro*, on the first larval stages of gastrointestinal nematodes of cattle, obtaining mortality of 19.4 %. Vieira and Cavalcante (1999) tested *A. squamosa in vivo* on gastrointestinal nematodes in goats. The plant reduced by 40% the count of *H. contortus* eggs in feces. With respect to adult forms of the parasites, *A. squamosa* showed reduction rates in the population of *H. contortus* and *Trichostrongylus columbriformis* of 21.8 % and 31.4 %, respectively, however not reducing the *Strongyloides papillosus* population. Yet according to the authors, the extract showed still to be effective against the adult form of *Oesophagostomum columbianum*, reducing by 74 % the parasites. The acute toxicity of plants from the Annonacea family is still poorly studied and research approaches its *in vitro* cytotoxic efficacy and with possible emphasis on the anti-tumor effects (Vieira and Cavalcante, 1999).

Annona muricata L. (Annonaceae), popularly known as graviola, is a medium -sized fruit tree commonly found in the tropics. The species has been widely used in folk medicine as an anthelmintic, antipyretic, sedative, antispasmodic, and anticonvulsant and as a hypotensive agent in humans (Costa et al., 2002). *In vitro* tests to evaluate the inhibition of egg hatching, larval and adult worm motility are widely used in prospecting for new anthelmintic agents (Vasconcelos et al., 2007).

Ferreira et al. (2013), researching *H. contortus* in sheep, demonstrated that aqueous extract of *A. muricata* leaves at 50, 25, 12.5 and 6.25% concentrations inhibited larval hatching in 84, 9 , 79, 1, 66, 9 and 47.42 %, respectively. The authors also evaluated the effect on the motility of L3 *H. contortus* larvae at the same concentrations and obtained reduction motility rates at 83.29 %, 89.08 %, 74.62 % and 30.47 %, indicating significant activity of *A. muricata* on infective larvae of this parasite. However, when were evaluated the activity of the extract on the motility of adult parasites, the response was not dependent on dosage, being able to observe the extracts activity at different concentrations within the first six hours of exposure. Phytochemical analysis did not reveal any type of acetogenins or even alkaloids in the extract but indicated the presence of phenolic compounds in the aqueous leaf extract of *A. muricata* (Ferreira et al., 2013)

Furthermore, since acetogenins have been associated with neurodegeneration in rats and in humans (Champy et al., 2004) the absence of acetogenins in the *A. muricata* extract is a somewhat of a motivating fact, because it can make for this aqueous extract a safe drug to treat targeted animals if compared with plant extracts prepared with organic solvents presuming the extraction of acetogenin (Ferreira et al., 2013).

Annona crassiflora Mart. (Annonacea) commonly known as “panã”, “araticum”, “cabeça de negro” , “cascudo”, “cortiça”, “marolo” ou “pinha do cerrado”, stands out due to the fruit’s flavor, and is used in alternative medicine for possessing antibacterial and antifungal properties (Almeida et al., 1998). It is characterized by being a timber tree species, deciduous in the dry season, hermaphrodite and xerophytic. The phenology of this species is established by flowering early in the rainy season, which occurs from September to December, with fruiting having started in November, with ripened fruit from January to March (Lorenzi and Matos, 2002). The fruits are used as food and appreciated for having a sweet and yellowish pulp with a strong aroma (Roesler et al., 2007).

Queiroz et al. (2012), using ean thanol extract from the leaves of *A. crassiflora* verified the action of this extract on *H. contortus* larval development in sheep at 100 and 50 mg/mL^{-1} mg/mL^{-1}. The authors also obtained an anthelmintic efficacy superior to 98.6 % for the larval development of *H. contortus*, using dried leaves of the same plant at a coproculture concentration of 333.3 mg (ms)/mL^{-1}. The aqueous extract from the seeds and leaves of *A. crassiflora* showed anthelmintic efficacy of 99.43 % and 89.81 %, respectively at 100 mg/mL^{-1} (Nogueira, 2009), presenting a promising alternative for the control of *H. contortus* in sheep.

In Southeastern region of Brazil, and especially in the North and Northeast, the “cajazeira” (*Spondias mombin* L.) also known as “caja-mirim”, “ambaró”, “taperebá”, is a fruit species belonging to the Anacardiaceae family. Utilized as source of permanent shading for the cocoa tree, it is also utilized by producing fruits that serve as an important source of additional income for the producer. The fruits' juicy, yellow, sour and aromatic properties are appreciated in refreshments and liquors (Sacramento, 2000). The use of the "cajazeira" in folk medicine and by the pharmaceutical industry has increased, being utilized in the treatment of fevers, as an antidiarrheal, antidesintéric, antiblenorrágic and anti-hemorroidiary. According Sacramento (2000), research has recently revealed that the leaf extract contains ellagic tannins giving the plant antiviral properties. Ademola et al. (2005), using the *S. mombin* aqueous and ethanol extract against *H. contortus*, obtained a reduction of approximately 65% of eggs found in the sheep feces (OPG) at a 500 pc mg/Kg^{-1} concentration.

Lippia sidoides Cham. (Verbenaceae) or alecrim pimenta is a species often used as herbal medicine in Northeast of Brazil, due to the antiseptic action owing to the high levels of thymol and carvacrol (Matos and Oliveira, 1998). According Camurça-Vasconcelos et al. (2007) and Vasconcelos (2006), the essential oil of *L. sidoides* possess an inhibitory effect in vitro on *H. contortus* eggs in sheep at 0.02 mg/mL^{-1} to 1.25 mg/mL^{-1}, respectively. Souza et al. (2010), Bevillaqua et al*.* (2005), and Person (2001), obtained same results using this oil at 0.5% and 1%, respectively. In tests conducted *in vivo*, Camurça-Vasconcelos et al. (2008), reported an efficacy of 54% from the oil of *L. sidoides* in the control of *H. contortus* in sheep at a 283 mg/Kg^{-1}, 14 days after treatment.

The genus *Caryocar*, one of the representatives of the family Caryocaraceae family, has 16 species that are found in South and Central America (Maya et al., 2008). *Caryocar brasiliense* Cambess. specie is a tree species native to the Cerrado regions with wide distribution in the Southeast and Midwest of Brazil (Maia et al., 2008). The popular name of this plant species may vary according to the region of occurrence, the most common being: "Pequi", "Piqui","piquiá-bravo", "amêndoa de espinho", "grão de cavalo", "pequiá", "pequiá-pedra", "pequerim", "Suari"and "piquiá" (Santos et al. 2004). Fruiting is annual and harvesting occurring in the period lasting from September to February (Vera et al*.*, 2005).

The aqueous extract from the *C. brasiliense* fruit peels, at 200 mg/$mℓ^{-1}$, significantly inhibited the development of *H. contortus* larvae in sheep. The plant extracts effectiveness in the inhibition of larval development was of 94.8%. The egg-hatching inhibition of LC50 and LC90 was of 23.82 and 53.19 mg/mL^{-1}, respectively. The qualitative phytochemical tests performed in this study indicated the presence of catechins, steroids, flavonoids, saponins, total tannins, xanthones and tannins catechetical (Nery, 2009).

Nogueira et al. (2012) evaluated the aqueous extract of *C. brasiliense* fruit's skin in the egg hatching inhibition test, with concentrations at 15 and 7.5 mg/ml^{-1}, reported anthelminthic efficacy corresponding to 98.7 % and 91.8 %, respectively. For these concentrations, the average L1 were significantly lower than treatment with distilled water or albendazole. The average for unembryonated eggs observed in all the treatments by extract was not different from the distilled water control and suggests that "Pequi" metabolites do not inhibit the embryogenesis of these nematodes, while they may reduce hatching. The egg-hatching inhibition of LC50 and LC90 were 3.81 and 7.35 mg/mL^{-1}, respectively.

In vivo, the average fecal egg count observed for the groups treated with the aqueous extract fruit peels of "Pequi" differed from the untreated group at concentration 2 g/Kg^{-1} bw. During the first and second weeks of post treatment, it was observed a 33 and 32.2 % of anthelminthic efficacy *in vivo*, respectively, compared to pretreatment when all animals showed high levels of infection (Nogueira et al*.*, 2012).

The crude powder derived from the "Pequi" fruit peels and leaves showed high efficiency (superior to 90 %) for the inhibition of larval development (LPGF) of *H. contortus* in sheep. The average LPGF for the concentrations at 250, 200 and 150 mg/mL were statistically similar to those observed for the control with the commercial anthelmintic. The aqueous extract from the leaves of the "Pequi" showed higher anthelmintic action within seven days of incubation. The lethal concentrations of LC50 and LC90 after seven days of incubation were 34.95 and 79.74 mg/mL, respectively, for the crude powder of the fruit peels and 69.05 and 97.19 mg/mL for the crude powder from the leaves. For the aqueous extract of the leaves, the LC50 and LC90 were 56.36 and 115.65 and mg/mL^{-1}, respectively (Fonseca, 2012).

Morais-Costa et al. (2012) compared the efficacy of *C. brasiliense* from the northern and central region of Minas Gerais in Brazil. For both regions, the concentration 333.33 mg/mL^{-1} of dried leaves of *C. brasiliense* showed higher efficacy than negative control with distilled water and showed anthelmintic activity similar to the control with ivermectin (16 mg/mL^{-1}). The dried leaves of this plant from northern and central region had anthelmintic action with efficacy of 98.52 % and 83.09 % respectively. This difference could be related to vegetation/area where the species were collected, since the area of vegetation in the northern region is a native and preserved area, which favors better performance and establishment of plant species in the Cerrado.

The species *Anacardium occidentale* L. belonging to the Anacardiaceae family, is popularly known as cashew tree (cajueiro). It is native to Brazil and used in traditional medicine, especially in northeastern Brazil due to its therapeutic effects. In the literature, there are proven pharmacological activities, as the cajueiro being anti-inflammatory plant (Olajide, 2004), ant diabetic (Barbosa-Filho et al., 2005), inhibitor of acetylcholinesterase (Barbosa-Filho et al., 2006) and antimicrobial (Akinpelu, 2001). Aiming to evaluate the anti-parasitic activity of *Anacardium humile* A. St. - Hil. (Anacardiaceae). Nery et al. (2010), used aqueous and ethanolic extracts of leaves against different species of gastrointestinal nematodes in sheep. The aqueous extract anthelmintic activity showed significantly higher than negative control at all concentrations. At concentrations of 150 and 187.5 mg/mL^{-1}, the percent efficacy was not significantly different from ivermectin (positive control, 16 mg/mL^{-1}). The LD50 in the inhibition assay for larval development was 10.14 mg/mL^{-1}, and for the 5 % confidence interval it was 13.36-6.83 mg/mL^{-1}. Results of the ethanolic extract were not significantly different from ivermectin at 60 mg/mL^{-1}. The LD50 was mg/mL^{-1} 23.24. Larvae of *Haemonchus* spp. (68%), *Strongyloides* spp. (31%) and *Trichostrogylus* spp. (1%) were identified in the coprocultures of the negative control group. This suggests that the extracts were effective against the three nematodes considered to be the most prevalent and pathogenic in sheep (Ueno and Gonçalves, 1998).

Morais-Costa et al. (2012), in preliminary study, the activity of anthelmintic was evaluated to *Paullinea* sp. on gastrointestinal nematodes of sheep. The leaves of this plant were collected in the city of Montes Claros, Brazil. In this study, *Paullinea* sp. at 333.3 mg/mL^{-1} differed from the treatment with distilled water and showed anthelmintic activity of 70.12 %, similar to treatment with ivermectin. In the control group, 100% of larvae were *Haemonchus* sp.. The anthelmintic activity of the Sapindaceae family and the species *Paullinea* sp. may be associated with saponin and tannin respectively.

The "genipapo" (*Genipa americana* L.), Rubiaceae family, tree that has been used in folk medicine, foods and animal feed , leather tanning, forestry, and by logging industries. The species, native to South America, has ecological importance, and is suitable for planting in degraded areas and wetlands (Epistein, 2001). In the egg hatching inhibition test, the aqueous extract of *G. american* leaves at 100 mg/mL, completely inhibited hatching. The relative average number of embryonated eggs was significantly greater than those of unembryonated eggs at 75 and 100 mg/mL^{-1}. This observation suggests a greater efficacy in inhibiting hatching rather than interfering with embryo development. The LC50 and LC90 of aqueous extract from *G. American* leaves were 34.3 and 79.8 mg/mL^{-1}, respectively. In the larval development inhibition test, concentrations ≥ 30 mg/mL^{-1} showed anthelminthic efficacy above 94 %. The LC50 and LC90 mg/mL^{-1} were 14.6 and 28.7, respectively (Nery, 2009). This suggests that the extracts were effective against the several nematodes considered to be the most prevalent and pathogenic in sheep (Wood et al., 1995), showing a wide spectrum of action. However, using the hydro-alcoholic extract from the leaves of "genipapo", Krychak-Furtado (2006) found 100 % efficacy for EHI at 50 mg/mL^{-1}, thus suggesting the metabolites extracted with alcohol could also show action against nematode eggs.

In an experiment conducted by Costa (2010), the species *Schinopsis brasiliensis* Engl. (Anacardiaceae), *Baccharis tridentata* Vahl . (Asteraceae), *Ximenia americana* L. (Olacaceae), *Lippia sidoides* Cham. (Verbenaceae), *Paullinea* sp . (Sapindaceae) were selected by ruminants in the Cerrado, which are considered to be anthelmintic and tanniferous. The animals showed no worm problems during this research, but there is a need for *in vitro* and *in vivo* to better evaluate the effectiveness of these species.

It was reported by Morais-Costa et al. (2012) at a 333.3 mg/mL, the effectiveness of the dried leaves derived from the plant species *Evolvulus* sp. (Convolvulaceae), *Acosmium dasicarpum (*Vogel) (Fabaceae Faboideae) *Heteropterys byrsonymifolia* A. Juss. (Malphigiaceae), *Lippia sidoides* Cham. (Verbenaceae), *Erytroxylum deciduum* A.St.-Hil. (Erythroxylaceae), *Senna spectabilis* (DC.) HSIrwin & Barneby (Fabaceae), *Baccharis tridentata* Vahl. (Asteraceae), *Casearia sylvestris* Sw (Salicaceae), *Paullinea* sp. (Sapindaceae), *Piptadenia viridiflora* (Kunth) Benth (Fabaceae) and *Ximenia americana* L. (Olacaceae). After the logarithmic transformation and variance analysis, it was found that the average LDPG for treatments with the dehydrated leaves, did not differ from the control by ivermectin, and the efficacies were: *X. Americana* (99.84%), *P. viridiflora* (85.77%), *Paullinea* sp. (70.12%) and *C. sylvestris* (43.63%). This effect can be attributed to condensed tannin concentrations at 10 mg of ethanol extracts from *C. sylvestris* (7.36%) *Paullinea* sp. (6.37%), *P.viridiflora* (1.75%) and *X. Americana* (0.36%). This study demonstrates a potent anthelmintic activity *in vitro*, for the ethanol extract. The fact that some species of this study have low condensed tannin content highlights the synergism among chemical compounds.

Final Considerations

It is necessary to scientifically validate new alternative anthelmintic compounds, characterizing them in the control of ruminant GNIs, and to evaluate the toxicity of these compounds, *in vivo* experiments should be performed, providing some of the plant species to the animals. Thus, species rich in tannins, catechetic tannins, catechins, steroids, flavonoids, xanthones and saponins have promising potential in the control of nematodes of ruminants and furthermore, can be active in synergism of these metabolites.

The species *Anacardium occidentale, Annona crassiflora, A. muricata, A. squamosa, Caryocar brasiliensis, Chenopodium ambrosioides, Genipa americana Lippia sidoides, Paullinea* sp., *Piptadenia viridiflora, Spondias monbin and Ximenia Americana* are adapted to the Cerrado and showed very promising results in reducing the bioactivity in the development of gastrointestinal nematodes of ruminants in Brazil.

In order to clarify the mechanisms of action from extracts of plant species, on the development of larvae using an electronic microscopy, would be a tool to support the study on reducing the use of chemical products, favoring lower incidences of residues in products of animal origin, thereby reducing costs and environmental impacts of these products in the environment.

Plant species rich in tannins, saponins and other secondary compounds, are deserving of further accurate studies to prove the scientific efficacy in controlling gastrointestinal parasites. Therefore, few studies have evaluated the metabolic anthelmintic effect of plant species from the Cerrado as well as possible toxicity effects. Thus, emerges the need to use natural products, based on plant species that are naturally selected by ruminants in this biome.

References

Ademola, IO; Fagbemi, BO; Idowu, SO. Anthelmintis activity of extracts of *Spondias mombin* against gastrointestinal nematodes of sheep: studies in vitro and in vivo. *Tropical Animal Health and Production*, v. 37, n. 3, 223-35, 2005.

Akinpelu, DA. Antimicrobial activity of *Anacardium occidentale* bark. *Fitoterapia*, v. 72, 286-287, 2001.

Almeida, SP. *Frutas nativas do cerrado: caracterização físico-química e fonte potencial de nutrientes*. *In* Cerrado: ambiente e flora. Embrapa-CPAC, Planaltina, 1998, 247-281.

Amarante, AFT; Barbosa, MA; Oliveira, MAG; Carmello, MJ; Padovani, CR. Efeito da administração de oxfendazol, ivermectina e levamisol sobre os exames coproparasitológicos de ovinos. *Brazilian Journal of Veterinary Research and Animal Science*. São Paulo, v. 29, n. 1, 31-38, 1992.

Amorim, A; Rodrigues, MLA; Borba, HR. Influência de extratos vegetais *in vitro* na viabilidade de larvas de nematóides gastrointestinais de bovinos. *Revista Brasileira de Farmácia* , v. 77, 47-48, 1996.

Athanasiadou, S; Kyriazakis, I. Plant secondary metabolites: antiparasitic effects and their role in ruminant production systems. *Proceedings of the Nutrition Society*, v. 63, n. 4, 631-639, 2004.

Athanasiadou, S; Kyriazakis, I; Jackson, F; Coop, RL. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Veterirany Parasitology*, v. 99, 205-219. 2001.

Barbosa-Filho, JM; Medeiros, KCP; Diniz, MFFM; Batista, LM; Athayde-Filho, PF; Silva, MS; Da-Cunha, EVL; Almeida, JRGS; Quintans-Júnior, LJ. Natural products inhibitors of the enzyme acetylcholinesterase. *Revista Brasileira de Farmacognosia*, v. 16, 258-285, 2006.

Barbosa-Filho, JM; Vasconcelos, THC; Alencar, AA; Batista, L; Oliveira, RAG; Guedes, DN; Falcão, HS; Moura, MD; Diniz, MFFM; Modesto-Filho, J. Plants and their active constituents from South, Central, and North America with hypoglycemic activity. *Revista Brasileira de Farmacognosia*, v. 15, 392-413, 2005.

Bevilaqua, CML; et al. Ovicidal and larvicidal activity of *Lippia sidoides* and *Ocimum gratissimum* essencial oils against *Haemonchus contortus*. Proceedings oft the 20th International conference of the World Association for the Advancement of Veterinary Parasitology, Christchurch. *New Zealand Veterinary Journal*, v.1, 78-9, 2005. Disponível em: <http//www.sci quest.org.nz.pdf>. Acesso em: 26 agosto 2013.

Bizimenyera, ES; Githiori, JB; Eloff, JN; Swan, GE. In vitro activity of *Peltophorum africanum* Sond. (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *Trichostrongylus colubriformis*. *Vet Parasitol*, v. 142, 336-343, 2006.

Calderón-Quintal, JA; Torres-Acostaa, JFJ; Ca Sandoval Castroa, CA; Alonsob, MA; Hoste, H; Aguilar-Caballero, A. Adaptation of *Haemonchus contortus* to condensed tannins: can it be possible? *Arch Med Vet*, v. 42, 165-171, 2010.

Camurça-Vasconcelos, ALF; et al. Anthelmintic activity of *Lippia sidoides* essential oil on sheep gastrointestinal nematodes. *Veterinary Parasitology*, v. 154, 167-70, 2008.

Castro, AG. *Defensivos agrícolas como um fator ecológico*. Jaguariúna. EMBRAPA - CNPDA. Documento, 6, 1989.

Champy, P; Höglinger, GU; Feger, J; Gleye, C; Hocquemiller, R; Laurens, A; Guerineau, V; Laprevote, O; Medja, F; Lombes, A; Michel, PP; Lannuzel, A; Hirsch, EC; Ruberg, M. Annonacin, a lipophilic inhibitor of mitochondrial complex I, induces nigral and striatal neurodegeneration in rats: possible relevance for atypical parkinsonism in Guadeloupe. *J Neurochem*, v. 88, 63-69, 2004.

Chang, FR; Wu, YC. Novel cytotoxic annonaceus acetogenins from *Annona muricata*. *Journal of Natural Products*, v. 64, 925-931, 2001.

Costa, CTC; Morais, SM; Bevilaqua, CML; Souza, MMC; Leite, FKA. Ovicidal effect of *Mangifera indica* L. seeds extracts on *Haemonchus contortus*. *Brazilian Journal of Veterinary Parasitology*, v. 11, 57-60, 2002.

Costa, FM. Influência da estrutura da vegetação na seleção da dieta de ovinos em pastejo, em área de cerrado. Montes Claros, 2010. 78p. Dissertação (Mestrado). Universidade Federal de Minas Gerais/Instituto de Ciências Agrárias, 2010.

Eiten, G. Vegetação do cerrado. In: PINTO, M. N. (Org.). *Cerrado - caracterização*, *ocupação e perspectivas*. Brasília, DF. Editora da Universidade de Brasília, Brasília, 1993, 17-73.

Epistein, L. Cultivo e aproveitamento do jenipapo. *Rev Bahia Agrícola*, v. 4, 23-24, 2001.

Felfili, JM; Fagg, CW; Silva, JCS; Oliveira, ECL; Pinto, JRR; Silva-Júnior, MC; Ramos, KMO. *Plantas da APA Gama e Cabeça de Veado:* espécies, ecossistemas e recuperação. Brasília: Universidade de Brasília, DF. Departamento de Engenharia Florestal, 2002, 52 p.

Ferreira, LEA; Castro, PMN; Chagas, ACS; França, BSC; Beleboni, ARO. In vitro anthelmintic activity of aqueous leaf extract of *Annona muricata* L. (Annonaceae) against *Haemonchus contortus* from sheep. *Experimental Parasitology*, v. 134, 327-332. 2013.

Fonseca, LD. Potencial antihelmíntico de *Caryocar brasiliense* Cambess. (Caryocaraceae) no controle de nematódeos gastrintestinais de ruminantes. Montes Claros, 2013. 92p. Dissertação (Mestrado). Universidade Federal de Minas Gerais/Instituto de Ciências Agrárias, 2013.

Fortes, E. *Parasitologia veterinária*. Porto Alegre: Sulina, 606p., 1993.

Geum-Soog, K; Zeng, L; Alali, F; Rogers, LL; Wu, F; Mclaughlin, JL; Sastrodihardjo, S. Two new mono-tetrahydrofuran ring acetogenins, annomuricin E and muricapentocin, from the leaves of Annona muricata. *Journal of Natural Products*, v. 61, 432-436, 1998.

Gopal, RM; Pomroy, WE; West, DM. Resistance of field isolates of *Trichostrongylus colubriformis* and *Ostertagia circumcincta* to ivermectin. International. *Journal for Parasitology*, v. 29, 781-786, 1999.

Hammond, JA; Fielding, D; Bishop, SC. Prospects for plant anthelmintcs in tropical veterinary medicine. *Vet Res Commun*, v. 21, 213-228, 1997.

Hoste, H; Jackson, F; Athanasiadou, S; Thamsborg, SM; Hoskin, SO. The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends Parasitol*., v. 22, 253-261, 2006.

Kahn, LP; Diaz-Hernandez, A. *Nutrition: Proceedings of an international conference*. Canberra: Australian Centre for International Agricultural Research. Chapter 5, Tannins with anthelminthic, 2000, 130−139.

Ketzis, JK. et al. Evaluation of efficacy expectations for novel and non-chemical helminth control strategies in ruminants. *Veterinary Parasitology*, v.139, 321-335, 2006.

Ketzis, JK; Taylor, A; Bowman, DD; Brown, DL; Warnick, LD; Erb, HN. *Chenopodium ambrosioides* and its essential oil as treatments for *Haemonchus contortus* and mixed adult-nematode infections in goats. *Small Ruminantes Research*, v. 44, 193-200, 2002.

Klink, CA; Machado, RBA. conservação do cerrado brasileiro. *Megadiversidade*, Belo Horizonte, v. 1, 147-145, 2005.

Krychak-Furtado, S. Alternativas fitoterápicas para o controle da verminose ovina no estado do Paraná: testes in vitro e in vivo. 2006. 147 f. Tese (Doutorado em Agronomia) - Departamento de fitotecnia e fitossanitarismo. Universidade Federal do Paraná, Curitiba, 2006.

Lorenzi, H; Matos, FJA. *Plantas medicinais no Brasil:* nativas e exóticas cultivadas. Nova Odessa: Instituto Plantarum, 2002. v. 2.

Maia, JGS; Andrade, EHA; Silva, MHL. Aroma volatiles of pequi fruit (*Caryocar brasiliense* Camb.). *Journal of Food Composition and Analysis*, v. 21, 574-576, 2008.

Miranda, IS; Almeida, SS; Dantas, PJ. Florística e estrutura de comunidades arbóreas em cerrados de Rondônia, Brasil. *Acta Amazônica*, v. 36, 419-430, 2006.

Mittermeier, N; Myers, RA; Mittermeier, CG. *Hotspots*: earth's biologically richest and most endangered terrestrial ecoregions. Mexico: CEMEX, 1999, 430p.

Molento, MB; Prichard, RK. Effect of multidrug resistance modulators on the activity of ivermectin and moxidectin against selected strains of Haemonchus contortus infective larvae. *Pesq Vet Bras*, v. 21, 117-121, 2001.

Morais-Costa, F; Queiroz, IR; Vasconcelos; Ferreira, AVP; Costa, MAMS; Vieira, TM; Martins, MAD; Duarte, ER; Lima, WS. Efficacy of *Paullinea* sp. (SAPINDACEAE), in the alternative control of gastrointestinal nematodes, Minas Gerais state, Brazil. In: ATBC 2012. Bonito/MS., 2012.

Morais-Costa, F; Cruz, ALMC; Duarte, ER; Lima, WS. Potencial antihelmíntico de espécies vegetais do cerrado, na inibição do desenvolvimento larval de *Haemonchus* spp. In: Encontro de Parasitologia, 2012. Belo Horizonte/MG., 2012.

Morais-Costa, F; Queiroz, IR; Vasconcelos, VO; Fonseca, DL; Ferreira, AVP; Costa, MAMS; Vieira, TM; Mota, GS, Duarte, ER; Lima, WS. Eficácia de Caryocar brasiliense cambess. (Caryocaraceae) de diferentes regiões de minas gerais, no controle alternativo de nematódeos gastrintestinais. In: Congresso de Parasitologia, 2012, São Luiz/MA., 2012.

Morton, JF. Sugar Apple. In: *Fruits of warm climates*, 69-72, 1987.

Nery, PS. Eficácia de extratos vegetais no controle da helmintose ovina, no Norte de Minas Gerais. Montes Claros, 2009. 102p. Dissertação (Mestrado). Universidade Federal de Minas Gerais/Instituto de Ciências Agrárias, 2009.

Nery, PS; Nogueira FA; Martins, ER, Duarte, ER. Effects of *Anacardium humile* leaf extracts on the development of gastrointestinal nematode larvae of sheep. *Veterinary Parasitology*, v.171, 361-364, 2010.

Nimer, E. *Climatologia do Brasil*. Rio de Janeiro: IBGE, 1989, 421 p.

Nogueira, FA, Nery, PS, Ferreira, M; Duarte, ER; Martins, ER. Plantas Medicinais no Controle Alternativo de Verminose em Ovinos. *Rev*. *Bras*. *de Agroecologia*, v. 4, n. 2, 2009.

Nogueira, FA; Fonseca, LD; Silva, RB; Ferreira, AVP; Nery, PS; Geraseev, LC; DuartE, ER. In vitro and in vivo efficacy of aqueous extract of *Caryocar brasiliense* Camb. to control gastrointestinal nematodes in sheep *Parasitol Res*, p. 111, 325-330, 2012.

Olajide OA. Effects of *Anacardium occidentale* stem bark extract on in vivo inflammatory models. *Journal of Ethnopharmacology*, v. 95, 139-142, 2004.

Oliver-Bever, B. *Medicinal plants in tropical west Africa*. *III Anti-infection therapy with higher plants*, v. 9, n. 1, 1983, 1- 85.

Queiroz, IR; Bastos, GA; Ferreira, AVP; Costa, FM; Duarte, ER; Oliveira, NJF. Eficácia de *Annona crasiflora* Mart. (Annonaceae) na inibição do desenvolvimeto larval de nematódeos gastroinstestinais de ovinos. In: XIV Semana da Biologia. 2012. Montes Claros/MG., 2012.

Queiroz, IR; Morais-Costa, F; Vascocelos, VO; Vieira, TM; Fonseca, LD; Ferreira, AVP; Costa, MAMS; Bastos, GA; Duarte, ER; Oliveira, NJFO. uso de *Annona crassiflora* (Annonaceae) no controle de nematóides gastrointestinais de ovinos. In: *Congresso de Parasitologia*. 2012. São Luiz/MA., 2012.

Reatto, A; Correia, JR; Spera, ST. Solos do bioma cerrado. In: SANO, S. M.; ALMEIDA, S. P. (Ed). *Cerrado*: ambiente e flora. Planaltina Embrapa/CPAC, 1998, 47-86.

Ribeiro, JF; Walter, BMT. As principais fitofisionomias do Bioma Cerrado. In: SANO, S. M.; ALMEIDA, S. P.; RIBEIRO, J. F. *Cerrado*: ecologia e flora. Brasília, DF, Embrapa Cerrados, 2008, 279 p.

Sacramento, CK; Souza, FX. de. *Cajá* (*Spondias mombin L*.). Jaboticabal: FUNEP, 2000, 42p

Sales, HR; Santos, RM; Nunes, YRF; Morais-Costa, F; Souza, SCA. Caracterização florística de um fragmento de cerrado na APA Estadual do Rio Pandeiros – Bonito de Minas/MG. *MG*. *Biota*, Belo Horizonte, v. 2, 22-30. 2009.

Sales, HR; Souza, SCA; Luz, GR; Morais-Costa, F; Amaral, VB; Santos, RM; Veloso, MDM; Nunes, YRF. Flora arbórea de uma floresta estacional decidual na APA Estadual do Rio Pandeiros, Januária/MG. *MG*. *Biota*, Belo Horizonte, v. 2, 31-41, 2009.

Sano, SM; Almeida, SP; Ribeiro, JF. (Org.). *Cerrado*: ecologia e flora. Brasília-DF: Embrapa Informação Tecnológica, 2008, v. 2.

Santos, BR; Paiva, R; Dombroski, JLD; Martinotto, C; Nogueira, RC; Silva, AAN. *Pequizeiro (Caryocar brasiliense Camb.): uma espécie promissora do cerrado brasileiro*. Lavras: Editora Ufla, 2004 (Boletim Técnico).

Silva, GG; Souza, PA; Morais, PLD; Santos, EC; Moura, RD; Menezes, JB. Caracterização do fruto de ameixa silvestre (Ximenia americana L.). *Rev*. *Bras*. *Frutic*., v. 30, 311-314, 2008.

Silva, RB; Rocha, FT; Morão, RP; Nogueira, FA; Marcelo, NA; Duarte, ER. Eficácia de levamizol e albendazol em rebanhos ovinos na região norte de Minas Gerais. In... 47ª Reunião Anual da Sociedade Brasileira de Zootecnia, 2010, Salvador, 2010.

Simões, CMO; Mentz, LA; Schenkel, EP; Irgang, BE; Stehmann, JR. *Plantas da Medicina Popular no Rio Grande do Sul*. *Porto Alegre*. 5º Edição. Ed. da Universidade/UFRGS, 1998, 42-5.

Souza, WMA; Ramos, RAN; Alves, LC; Coelho, MCOC; Maia, MBS. Avaliação *in vitro* do extrato hidroalcoólico (EHA) de alecrim pimenta (*Lippia sidoides* Cham.) sobre o desenvolvimento de ovos de nematódeos gastrointestinais (Trichostrongylidae). *Revista Brasileira de Plantas Medicinais*, Botucatu, v. 12, 278-281, 2010.

Taylor, MA; Learmount, J; Lunn, E; Morgan, C; Craig, BH. Multipleresistance to anthelmintics in sheep nematodes and comparison of methods used for their detection. *Small Ruminant Res*, p. 86, 67-70, 2009.

Thomaz-Soccol, V; Souza, FP; Cristina Sotomaior, C; Castro, EA; Milczewski, V; Mocelin, G; Pessoa E Silva, MC. Resistance of Gastrointestinal Nematodes to Anthelmintics in Sheep (*Ovis aries*) *Brazilian Archives of Biology and Technology*, v. 47, 41-47, 2004.

Ueno, H; Gonçalves, PC. *Manual para diagnóstico das helmintoses de ruminantes*. Tokyo. Japan International Cooperation Agency, Tokyo, 1998, p. 143.

Vasconcelos, ALC; Bevilaqua, CM; Morais, SM; Maciel, MV; Costa, CT; Macedo, IT; Oliveira, LM; Braga, RR; Silva, RA; Vieira, LS. Anthelmintic activity of *Croton zehntneri* and *Lippia sidoides* essential oils. *Veterinary Parasitology*, v. 148, 288-294, 2007.

Vasconcelos, ALCF. Avaliação da atividade antihelmíntica dos óleos essenciais de *Lippia sidoides* e *croton zehntneri* sobre nematoides gastrintestinais de ovinos. 2006. 83 f. Tese (Doutorado – Área de Concentração em Reprodução e Sanidade Animal) - Faculdade de Medicina Veterinária, Universidade Estadual do Ceará, Fortaleza, 2006.

Vera, R; Naves, RV; Nascimento, JL; Chaves, LJ; Leandro, WM; Souza, ERB. Caracterização física de frutos do pequizeiro (Caryocar brasiliense Camb.) no estado de Goiás. *Pesquisa Agropecuária Tropical,* v. 35, 71-79, 2005.

Vieira, LS. *Alternativas de Controle da Verminose Gastrintestinal dos Pequenos Ruminantes*. *EMBRAPA*, 10 p., 2003.

Vieira, LS; et al. Evaluation of anthelmintic efficacy of plants available in Ceará State, North East Brazil, for the control of goat gastrointestinal nematodes. *Revue de Medecine Veterinaire*, Toulouse, v.150, n. 5, 447-452, 1999.

Vieira, LS. *Fitoterapia da Amazônia: manual de plantas medicinais*. São Paulo: Agr. Ceres, 1992, 350 p.

Vieira, LS; Cavalcante, ACR; Pereira MF; Dantas, LB; Ximenes, LJF. Evaluation of anthelmintic efficacy of plants available in Ceará State, North-east Brazil, for the control of goat gastrointestinal nematodes. *Revue Médicine Véterinaire*, v. 150, 447-452, 1999.

Wang, Li-Quan; et al. Annonaceous acetogenins from the leaves of *Annona montana*. *Bioorganic & Medicinal Chemistry*, v.10, 561-565, 2002.

Wood, IB; Amaral, NK; Bairden, K; Duncan, JL; Kassai, T; Malone, JB; Pankavich Jr., JA; Reineche, RK; Slocombe, O; Taylor, SM; Vercruysse, J. World Association for the advancement of veterinary parasitology (W. A. A. V. P.) second edition of guidelines for

evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). *Veterinary Parasitology*, v. 58, 181-213, 1995.
Wolstenholme, AJ; Fairweather, I; Prichard, RK; Von Samson-Himmelstjerna, G; Sangster, NC. Drug resistance in veterinary helminthes. *Trends Parasitol*., v. 20, 469-476, 2004.
Wu, FE; Zeng, L; Gu, ZM; Zhao, GX; Zhang, Y; Schwedler, JT; Mclaughlin, JL; Sastrodihardjo, S; New bioactive monotetrahydrofuran annonaceous acetogenins, annomuricin C and muricatocin C, from the leaves of *Annona muricata*. *Journal of Natural Products*, v. 58, 909-915, 1995.
Zanetti, R. *Análise fitossociológica e alternativas de manejo sustentável da mata da agronomia*, *Viçosa*, *Minas Gerais*. Viçosa, UFV. Trabalho integrante do conteúdo programático da disciplina Manejo Sustentado de Florestas Naturais, 1994, 92 p.