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Original Article

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Atividade anti-helmíntica de *Cymbopogon citratus* sobre *Haemonchus contortus*

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Abstract

Parasitic nematodes are of major economic importance in livestock. An alternative for the control of parasites is phytotherapy. This study evaluated the efficacy of *Cymbopogon citratus* decoction (CcD), *C. citratus* essential oil (CcEo) and citral against *Haemonchus contortus* using *in vitro* egg hatch test (EHT) and larval development test (LDT) and an *in vivo* test using a *Meriones unguiculatus* (gerbil) model. The effect of 800 mg/kg CcEo was evaluated in gerbils that had been artificially infected with 5,000 third-stage *H. contortus* larvae. The effective concentrations required to inhibit 50% (EC50) of egg hatching were 0.46, 0.14 and 0.13 mg/mL for CcD, CcEo and citral, respectively. The EC50 values in the LDT were 5.04, 1.92 and 1.37 mg/mL for CcD, CcEo and citral, respectively. *H. contortus* population in the group treated with *C. citratus* essential oil was reduced by 38.5% ($P<0.05$) in comparison to the control group. These results suggest that it may be possible to use *C. citratus* essential oil to control of *H. contortus* parasite of small ruminant.

Keywords: Phytotherapy, anthelmintic, *Meriones unguiculatus*, *in vitro*, citral.

Resumo

O parasitismo por nematoides tem grande importância econômica no rebanho. Uma alternativa para o controle de parasitas é a fitoterapia. Este estudo avaliou a eficácia do decocto de *Cymbopogon citratus* (DCc), do óleo essencial de *C. citratus* (OECc) e do citral contra *Haemonchus contortus* utilizando o teste *in vitro* de eclosão dos ovos (TEO) e o teste de desenvolvimento larval (TDL) e um teste *in vivo* com modelo *Meriones unguiculatus* (gerbil). O efeito de 800 mg/kg de OECc foi avaliado em gerbils infectados artificialmente com 5000 larvas de terceira fase de *H. contortus*. As concentrações efetivas necessárias para inibir 50% (CE50) da eclosão dos ovos foram 0,46; 0,14 e 0,13 mg/mL para DCc, OECc e citral, respectivamente. Os valores da CE50 no TDL foram de 5,04; 1,92 e 1,37 mg/mL para DCc, OECc e citral, respectivamente. No grupo tratado com óleo de *C. citratus* a população *H. contortus* foi reduzida em 38,5% ($P < 0,05$) em comparação com o grupo controle. Estes resultados sugerem que pode ser possível a utilização de óleo essencial de *C. citratus* para controle de *H. contortus,* parasita de pequenos ruminantes.

Palavras-chave: Fitoterapia, anti-helmínticos, *Meriones unguiculatus*, *in vitro*, citral.

Introduction

Haemonchus contortus is one of the most abundant and prevalent gastrointestinal parasite in sheep and goats in Brazil (SOUZA et al., 2013), besides causing acute disease and high mortality in all classes of livestock. Control programs have relied heavily on the use of synthetic anthelmintics. However, the efficacy of anthelmintics is increasingly endangered by the development of resistance in nematode populations (MILLER et al., 2012). The intensification of the animal production system has led to an increasing demand for effective and low-cost anthelmintic drugs to control helminth diseases (ADEMOLA et al., 2004). These concerns have led to the search for and evaluation of alternative control methods (ATHANASIADOU et al., 2001).

One promising alternative has been to research and identify plant products with anthelmintic properties (MAPHOSA et al., 2010). The selection of candidate plants with nematicidal activity involves several experimental stages, including an initial screening using *in vitro* assays with free-living stages of *H. contortus*

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(CAMURÇA-VASCONCELOS et al., 2005), and subsequent *in vivo* evaluations (JESÚS-GABINO et al., 2010). *Meriones unguiculatus* (gerbil) has been proposed as a suitable rodent model for the study of host–parasite interactions during *H. contortus* infection because of the histological similarity between the abomasum of lambs and the stomach of gerbils (CONDER et al., 1990). The gerbil model is an economical, easy-to-use and suitable *in vivo* model for the testing of anthelmintic agents and has the advantages of requiring smaller product quantities and allowing greater standardisation than livestock studies (SQUIRES et al., 2010).

The selection of plants for evaluation as potential anthelmintics is based on surveys of popular accounts of the biological activity against nematodes. *Cymbopogon citratus,* family Poaceae, was reported in an ethnoveterinary study as having anthelmintic activity (RITTER et al., 2012). Infusions or decoctions of dry leaves have been utilised as stomachic, antifever, antiseptic, carminative and tranquilising (ARHOGHRO et al., 2012; BORRELLI & IZZO, 2000).

In vitro tests with the aqueous extract of *C. citratus* reduced the burden of Strongyloidea larvae in goats (ALMEIDA et al., 2003). Several investigations have shown that the essential oil of *C. citratus* possesses antibacterial (NAIK et al., 2010), antifungal (NGUEFACK et al., 2012), antioxidant (PEREIRA et al., 2009), anti-*Leishmania* (MACHADO et al. 2012) and insecticidal effects (KUMAR et al., 2013). These properties are attributed primarily to the major constituent of the essential oil, the citral, which is a natural combination of two isomeric aldehydes (Figure 1), neral (cis-citral) and geranial (trans-citral). Together these compounds represent approximately 65-85% of the essential oil (SADDIQ & KHAYYAT, 2010).

The objective of this study was to evaluate the effects of the decoction and essential oil of *C. citratus* and their major constituent, citral on *H. contortus* through two *in vitro* tests, the egg hatching and larval development assays. The effects of essential oil were also evaluated in an *in vivo* test using gerbils.

Materials and Methods

The care and handling of animals were in accordance with the Brazilian legislation for use of animals in experiment (BRASIL, 2008), and the protocol was approved by the Ethical Committee of Ceará State University (number: 09657334-1).

Obtaining the plant

C. citratus was collected in the Horto of medicinal plants of the Federal University of Ceará (3° 44' S, 38° 33' W). This plant was authenticated, and voucher specimen was deposited in the Herbarium Prisco Bezerra of the Federal University of Ceará and numbered as 46090 with botanical identification by Luiz Wilson Lima-Verde.

The fresh aerial parts of *C. citratus,* 2975 g, were subjected to hydrodistillation for 3 hours in a Clevenger-type apparatus to obtain the essential oil. For the production of the decoctions, the water remaining after hydrodistillation was collected directly
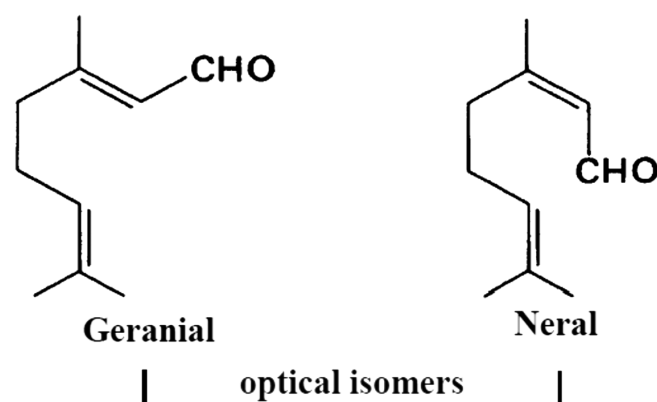

Figure 1. The chemical structures of geranial and neral (SILVA et al., 2009).

from the container of the apparatus. Subsequently, this water was filtered, frozen and lyophilised. The essential oil and decoction were stored at 4 °C until use. Citral (40:60 ratio of neral:geranial) was purchased from Sigma® (Chemical Company, St Louis, USA).

Egg hatch test

Sheep experimentally infected with *H. contortus* were used as a source of fresh eggs. The isolate of *H. contortus* is benzimidazole resistant and it was obtained from central region of the state of Ceará, Brazil. *H. contortus* eggs were recovered according to the method of Hubert & Kerboeuf (1992). The egg hatch test (EHT) was performed based on the methodology described by Coles et al. (1992). To increase their aqueous solubility, the essential oil, decoction and citral were diluted in 3% Tween 80. An egg suspension (250 µL) containing approximately 100 fresh eggs was incubated with 250 µL of essential oil or decoction or citral at concentrations from 0.03 to 2.5 mg/mL for 48 h at 25 °C. Drops of Lugol's solution were added. The eggs and the first-stage larvae (L1) were counted under a microscope. This test had two controls: a negative containing the diluent (3% Tween 80) and a positive containing 0.025 mg/mL thiabendazole. Three repetitions with five replicates for each essential oil concentration and for each control were performed.

Larval development test

A larval development test (LDT) was performed using an aliquot of egg suspension obtained according to the method of Hubert & Kerboeuf (1992). The suspension was incubated for 24 h at 25 °C to obtain L1. Next, 1 mL of larval suspension containing approximately 250 L1 and 1 mL of essential oil or decoction or citral at concentrations of 0.62 to 10 mg/ml were incubated with 2 g of faeces from a nematode-free sheep for 6 days at room temperature. Then, the third-stage larvae (L3) were recovered according to the method of Roberts & O'Sullivan (1950) and counted under a microscope. This test had two controls, a negative containing 3% Tween 80 and a positive containing 0.008 mg/mL ivermectin. Three repetitions with five replicates for each concentration and for each control were performed.

In vivo test with gerbils

This test was based on the methodology described by Jesús-Gabino et al. (2010). Ten 5-week-old male and female gerbils with a body weight range of 25–35 g were kept in polypropylene boxes and fed with commercial feed (Labina®) and water *ad libitum*. The animals were immunosuppressed with 100 µL of hydrocortisone (Azium®, Schering-Plough Labs) intramuscularly for two consecutive days to promote a better parasite establishment. The gerbils were fasted feed for 24 hours to facilitate artificial infection.

To perform the experimental infection, third-stage *H. contortus* larvae (L3) were artificially exsheathed through contact with 0.187% sodium hydrochloride for a few seconds. When the majority of the larvae were exsheathed, they were washed with distilled water and centrifuged at 3,000 rpm for 3 min. The gerbils were infected orally with 5,000 exsheathed *H. contortus* larvae. Four days after infection, the ten gerbils were divided randomly into two groups (n=5) and treated orally for two days: G1: (negative control) 3% Tween 80; and G2: 800 mg/kg of *C. citratus* essential oil.

Eight days after infection, the gerbils were euthanised. A necropsy was performed to remove the stomach and obtain the larvae. The stomachs were opened and placed in contact with phosphate-buffered saline (PBS) for 4h to obtain the larvae in the gastric lumen. Subsequently, the stomachs were removed and incubated by 16h with 1% mucosal digestion solution (pepsin, hydrochloric acid and distilled water) to recover the larvae retained on the gastric mucosa. Thus, larvae from the gastric lumen and mucosa were collected, quantified and recorded to obtain the average recovery of parasites per group.

Chemical analysis of the essential oil

The chemical composition of the essential oil was determined by gas chromatography (GC) and mass spectrometry (MS). The essential oil was analysed on a Hewlett-Packard 5971 instrument using the following experimental conditions: DB-1–coated fused silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness); carrier gas – helium; injector temperature –220 °C; detector temperature –200 °C; and column temperature program – 35 –180 °C at 48 °C /min, then 180 –250 °C at 10 °C/min. For MS, the electron ionization was 70 eV. Compounds were identified by their GC retention time, expressed in terms of Kovat's index, which was calculated by the Van den Dool and Kratz equation using a hydrocarbon homologue series and by comparison of the test compound's mass spectra with those present in the National Institute for Standard Technology computer data bank (NIST; 62,235 compounds) and with published spectra (ADAMS, 2001).

Phytochemical analysis of the decoction

The major classes of secondary metabolites present in the decoction were characterized according to the method of Matos (2009). The chemical characterization was based on the addition of specific reagents to decoction aliquots and observing the changes in the solution's colour or precipitate formation. The following experiments were performed: the identification of phenolic compounds (precipitation reaction with ferric chloride), the naphthoquinone reaction (acid/base), the characterization of flavonoids (cyanidin reaction and sulfuric acid), the presence of triterpenes and steroids (Liebermann-Burchard reaction), alkaloids (precipitation reactions with Dragendorff and Mayer reagents) and the characterization of saponins (Lieberman-Buchard reaction and the rate of spume).

Statistical analysis

The effectiveness of each treatment in the EHT was determined based on the percentage of hatched larvae using the following formula: number of hatched larvae/(number hatched larvae + number eggs) × 100.

The inhibition percentage in the LDT was calculated based on the per cent reduction in the number of L3 recovered relative to the number recovered from the negative control group: (number of L3 in the control group - number of L3 in the treated group)/number of L3 in control group × 100.

The results of the *in vitro* tests were expressed as the per cent inhibition of egg hatching or larval development. The data were analysed using ANOVA and were compared using the Tukey test ($P<0.05$) in GraphPad Prism program 5.0. The effective concentrations that inhibited 50% (EC50) of egg hatching and larval development were determined by the probit method using SPSS 8.0 for Windows.

The reduction of the parasite burden in gerbils treated with the essential oil was estimated using the following formula: (mean recovered parasites in the control group - mean recovered parasites in the treated group)/ mean of recovered parasites in the control group x 100. The data were analysed using ANOVA and were compared using the Tukey test ($P<0.05$) in GraphPad Prism program 5.0.

Results

The mean effectiveness of *C. citratus* essential oil, decoction and citral obtained in the EHT are presented in Tables 1, 2 and 3, respectively. The essential oil and citral inhibited egg hatching at concentrations ≤ 0.62 mg/mL ($P < 0.05$).

Tables 4, 5 and 6 presents the mean efficacy of *C. citratus* essential oil, decoction and citral determined in the LDT, respectively. At a concentration of 10 mg/mL, citral had an efficacy similar to that of the positive control ($P > 0.05$). The effectiveness of 5 mg/mL essential oil was not significantly different from that of the positive control ($P > 0.05$).

The yield of *C. citratus* essential oil was 0,519%. The results obtained by gas chromatography indicated that the main constituents of the essential oil were geranial (57.3%) and neral (40.4%). Phytochemical screening showed the presence of condensed tannins, saponins and flavonoids in decoction.

The mean number of *H. contortus* larvae recovered at necropsy and the per cent reduction in the parasite burden due to the treatment are presented in Table 7. The *C. citratus* essential oil reduced in

Table 1. Mean efficacy ± standard error of *Cymbopogon citratus* essential oil on *Haemonchus contortus* egg hatching.

Concentrations (mg/mL)	Essential oil
1.25	99.5 ± 0.1A
0.62	98.4 ± 0.3A
0.31	84.1 ± 1.1B
0.15	40.6 ± 1.5C
0.07	23.5 ± 1.6D
Tween 80 (3%)	13.4 ± 1.3D
Thiabendazole (0.025mg/mL)	96.4 ± 0.4A
EC50*	0.14

Means with the same capital letters in the columns are not significantly different (P > 0.05). *EC50 is the effective concentrations that inhibited 50% of egg hatching.

Table 2. Mean efficacy ± standard error of *Cymbopogon citratus* decoction on *Haemonchus contortus* egg hatching.

Concentrations (mg/mL)	Decoction
2.5	97.4 ± 0.6A
1.25	81.5 ± 2.7B
0.62	64 ± 5.3C
0.31	26.2 ± 3.2D
0.15	16.7 ± 2.1D
Tween 80 (3%)	11.9 ± 0.4D
Thiabendazole (0.025mg/mL)	96.4 ± 0.4A
EC50*	0.46

Means with the same capital letters in the columns are not significantly different (P > 0.05). *EC50 is the effective concentrations that inhibited 50% of egg hatching.

Table 3. Mean efficacy ± standard error of citral on *Haemonchus contortus* egg hatching.

Concentrations (mg/mL)	Citral
0.62	100.0 ± 0.0A
0.31	90.2 ± 1.3A
0.15	42.7 ± 3.8B
0.07	18.8 ± 2.5C
0.03	5.1 ± 1.3D
Tween 80 (3%)	5.0 ± 0.4D
Thiabendazole (0.025mg/mL)	96.8 ± 0.6A
EC50*	0.13

Means with the same capital letters in the columns are not significantly different (P > 0.05). *EC50 is the effective concentrations that inhibited 50% of egg hatching.

Table 4. Mean efficacy ± standard error of *Cymbopogon citratus* essential oil on *Haemonchus contortus* larval development.

Concentrations (mg/mL)	Essential oil
10.0	99.9 ± 0.0A
5.0	90.8± 1.6A
2.5	72.2 ± 3.5B
1.25	21.9 ± 5.2C
0.62	3.8 ± 1.3D
Tween 80 (3%)	5.2 ± 1.8D
Ivermectin (0.008 mg/mL)	99.9 ± 0.0A
EC50*	1.92

Means with the same capital letters in the columns are not significantly different (P > 0.05). *EC50 is the effective concentrations that inhibited 50% of larval development.

Table 5. Mean efficacy ± standard error of *Cymbopogon citratus* decoction on *Haemonchus contortus* larval development.

Concentrations (mg/mL)	Decoction
10.0	69.2 ± 4.9A
5.0	51.8± 6.4B
2.5	30.8 ± 3.8C
1.25	10.3 ± 3.8D
Tween 80 (3%)	4.9 ± 1.9D
Ivermectin (0.008 mg/mL)	99.9 ± 0.0E
EC50*	5.04

Means with the same capital letters in the columns are not significantly different (P > 0.05). *EC50 is the effective concentrations that inhibited 50% of larval development.

Table 6. Mean efficacy ± standard error of Citral on *Haemonchus contortus* larval development.

Concentrations (mg/mL)	Citral
10.0	99.9 ± 0.0A
5.0	90.9± 1.1B
2.5	79.4 ± 2.4C
1.25	43.2 ± 2.7D
0.62	17.8 ± 1.5E
Tween 80 (3%)	6.3 ± 1.7F
Ivermectin (0.008 mg/mL)	99.9 ± 0.0A
EC50*	1.37

Means with the same capital letters in the columns are not significantly different (P >0.05). *EC50 is the effective concentrations that inhibited 50% of larval development.

Table 7. Mean efficacy and *Haemonchus contortus* burden (± standard error) in *Meriones unguiculatus* (gerbil) treated with 800 mg/kg of *Cymbopogon citratus* essential oil.

Treatments	*Haemonchus contortus* load
C. citratus oil	
Mean Worm burden	223.4±21.7A
Efficacy (%)	38.6
Negative Control	
Mean Worm burden	363.2±38.7B

Means with the same capital letters in the columns are not significantly different (P > 0.05).

38.5% the *H. contortus* burden. The mean *H. contortus* burden from gerbils treated with essential oil was 223.4, compared with 363.2 larvae recovered from the control group. The comparison of the results of the two groups showed significant difference (*P* < 0.05).

Discussion

There has been growing interest in the use of herbal medicines to control gastrointestinal parasites because of the problem of anthelmintic resistance in nematode populations and growing concern about the presence of drug residues in animal products (GITHIORI et al., 2006). The use of plants with anthelmintic properties has been considered a suitable approach to worm control,

particularly for resource-poor livestock keepers, because this approach has the potential to be sustainable (ASASE et al., 2005).

The choice of *C. citratus* was based on the numerous reports of the biological activity of this plant, which acts as an insecticide (KUMAR et al., 2013), a repellent (KAZEMBE & CHAURUKA, 2012), a molluscicide (OTARIGHO & MORENNIKEJI, 2012) and a nematicide against the pinewood nematode *Bursaphelenchus xylophilus* (BARBOSA et al., 2010) and the plant nematode *Meloidogyne incognita* (GUPTA et al., 2011). In a Brazilian study that evaluation the anthelmintic activities of plants, *C. citratus* was cited as one of the species with more promising results (NERY et al., 2009).

A previous *in vitro* study showed that *C. citratus* aqueous extract reduced the number of *H. contortus* larvae by 97% at a concentration of 224 mg/mL (ALMEIDA et al., 2003), a worse than that obtained in the current work in which the same efficacy was obtained at a concentration of 5mg/mL. The *C. citratus* decoction also had better results than other plants did. For example, decoctions of *Alpinia zerumbet*, *Tagetes minuta* and *Mentha villosa* had EC50 values of 0.96 mg/mL, 0.66 mg/mL and 0.5 mg/mL, respectively, in an egg hatch test (MACEDO et al., 2012).

Although there are few reports of the anthelmintic activities of decoctions, its use to extract active ingredients from plants mimics popular methods because decoctions are easier to prepare and are less toxic to manipulate (SCHUCH et al., 2008). However, the preparation of decoctions may alter many active substances due to the prolonged heating, and these changes are a considered limitation of decoctions (FALKENBERG et al., 2000). The existence of biologically active compounds with ovicidal and larvicidal effects on *H. contortus* in the decoction was verified, and these effects were observed even after heating for two hours. The phytochemical screening of the decoction revealed the presence of secondary metabolites such as condensed tannins and flavonoids, considered the chemical components responsible for the wide range of therapeutic activities of several medicinal plants (LEE et al., 2008; OLIVEIRA et al., 2011). The role of condensed tannins in many biological processes related to different nematode stages have been confirmed by *in vitro* studies (HOUNZANGBE-ADOTE et al., 2005). However, the *in vitro* activity of the decoction in the work can be resulting by the synergistic interaction among classes of compounds.

C. citratus essential oil was more efficient than the decoction and exhibited an efficacy superior to those of other essential oils tested previously. The EC50 values of *Eucalyptus globulus* essential oil were 8.3 and 6.9 mg/mL for egg hatching and larval development, respectively (MACEDO et al., 2009). The EC50 values of *Lippia sidoides* essential oil were 0.4 and 2.97 mg/mL for eggs and larvae, respectively (CAMURÇA-VASCONCELOS et al., 2007). The EC50 of *Mentha piperita* essential oil was 0.26 mg/mL for egg hatching (KATIKI et al., 2011). The capacity to reduce hatching and development could be epidemiologically important and it could help to modulate the risk of parasitism by limiting the contamination of pastures grazed by ruminants (MAX, 2010).

Plant essential oils are an important group of products and can be used as an alternative or adjunct to current antiparasitic therapies (ANTHONY et al., 2005). Citral was the major constituent of *C. citratus* essential oil and was effective against

H. contortus eggs and larvae. Therefore, citral is likely the active substance responsible for the anthelmintic activity. Citral has antibacterial (FISHER et al., 2007), antifungal (YAMASAKI et al., 2007) and insecticidal activities (YANG et al., 2005), and it is also effective against the phytonematodes *Bursaphelenchus xylophilus* and *Meloidogyne incognita* (BAUSKE et al., 1994; CHOI et al., 2007) and the larvae of the nematode *Anisakis simplex* (HIERRO et al., 2006). Citral has also been reported as a predominant compound in *Eucalyptus staigeriana* essential oil and may be responsible for the activity of that essential oil against the gastrointestinal nematodes of goats (MACEDO et al., 2010).

A common feature of plant volatiles is their hydrophobic nature, and several studies addressing the mode of action of such compounds have suggested the cell membrane as the primary target (BAKKALI et al., 2008). Transcuticular diffusion is a common mode of entry into helminth parasites for non-nutrient and non-electrolyte substances (EGUALE et al., 2007). It is easier for lipophilic anthelmintics to cross the external membrane of helminths than it is for hydrophilic compounds to do so (GEARY et al., 1999). Essential oils can interfere with nematode metabolism, inhibiting or disorganising vital functions from the initial stages of development onward, and can also furthermore interfere with mechanisms of locomotion due to the possible destructuring of the nervous system (OKA et al., 2000).

After promising *in vitro* results were obtained, the essential oil was evaluated *in vivo*. Among laboratory animal models, gerbils have been shown to be the most suitable because of their capacity to become infected with and maintain infections of small ruminant nematodes (CONDER et al., 1992). Gerbils are also a good model for evaluating the anthelmintic activity of new drugs against *H. contortus in vivo* before conducting studies in ruminants (KÖNIGOVA et al., 2008). Larval exsheathment is a critical part of the process of host infection by parasites. The use of sodium hypochlorite to exsheath *H. contortus* larvae may limit their infectivity in gerbils (CONDER & JOHNSON, 1996). Howerever, the present work demonstrated that even larvae exsheathed with hypochlorite can have high infectivity and can establish infections in the stomachs of gerbils treated with immunosuppressants resulting in nematode loads higher than those in others studies. The explanation for the high load of recovered parasites may be related to the large inocula of larvae and to the use of mucosa digestion, which allows a higher larvae recovery rate.

C. citratus essential oil had an efficacy similar to that found for the n-hexane extract of *Prosopis laevigata* when this extract was administered intraperitoneally to gerbils infected with *H. contortus* (JESÚS-GABINO et al., 2010). The treatment with 500 mg/kg of *Eucalyptus staigeriana* essential oil, three consecutive days, achieved a 46.4% reduction of *H. contortus* burden in *M. unguiculatus* (RIBEIRO et al., 2013). The results for *C. citratus* were better than those for 300 mg/kg and 1,000 mg/kg concentrations of the essential oil and ethanolic extract of *Artemisia annua*, respectively, administered to gerbils for five days (SQUIRES et al., 2011). A single treatment with 600 mg/kg or 1200 mg/kg orange oil reduced the *H. contortus* burden by 7 and 25%, respectively. The same doses administered for five days had efficiencies of 62 and 87%, respectively (SQUIRES et al., 2010).

The number of treatments can also influence the efficacy of the tested product. Thus, the moderate efficiency obtained with gerbils infected with *H. contortus* may be due to the dose (level or number) or to the rapid elimination of the drug, leading to an insufficient contact time with the parasites. Therefore, plants with moderate anthelmintic activity should still be considered. Although such plants may not be useful as stand-alone alternatives to anthelmintic drugs, they may still be valuable as part of an integrated approach specifically designed to achieve sustainable parasite control in ruminant production systems (GITHIORI et al., 2006).

C. citratus has already been reported to be an anthelmintic agent. Therefore, the current findings justify the use of this plant in folk medicine. *C. citratus* essential oil exhibited promising results. Further experiments using different dose levels should be performed. In addition, tests can be performed with the essential oil to confirm its anthelmintic activity in target species.

Acknowledgements

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