

HOW TO CITE:

Mota YTR, França AK de A, Dantas MVG, Macedo RCB de S, Batista JIL, Soares KM da P. et al. In vitro anthelmintic effect of aqueous extract of *Croton blanchetianus* leaf on sheep endoparasites. Rev. Contexto & Saúde. 2025;25(50):e16453.

ORIGINAL ARTICLE

IN VITRO ANTHELMINTIC EFFECT OF AQUEOUS EXTRACT OF *CROTON BLANCHETIANUS* LEAF ON SHEEP ENDOPARASITES

Yandra Thaís Rocha da Mota¹, Ana Karolinne de Alencar França²,
Marcus Vinicius Gomes Dantas³, Renata Cristina Borges da Silva Macedo⁴,
João Inácio Lopes Batista⁵, Karoline Mikaelle de Paiva Soares⁶,
Michele Dalvina Correia da Silva⁷, Ana Carla Diógenes Suassuna Bezerra⁸

Highlights:

- (1) Control of gastrointestinal endoparasites using plants.
- (2) The use of plant products brings environmental and economic benefits.
- (3) The aqueous extract is more than 80% effective in inhibiting gastrointestinal parasites.

ABSTRACT

The development of small ruminants has been hampered by infections related to gastrointestinal endoparasites. This problem arose due to the incorrect use of anthelmintics and inadequate management practices, which led to the emergence of parasite resistance. Given this, herbal medicine offers an alternative approach to parasite control. Among the various options, *Croton blanchetianus* stands out for its bioactive compounds and demonstrates antibacterial, antifungal, ectoparasiticide and anthelmintic properties. Thus, the objective of this study was to evaluate the in vitro ovicidal activity of the aqueous extract derived from *Croton blanchetianus* leaves. For this, fecal samples were collected directly from the rectal ampulla of naturally infected animals, without administration of antiparasitics, for a period of 90 days. When the egg count per gram of feces exceeded 2,000 eggs, these eggs were collected for hatchability testing. Simultaneously, phytochemical analyzes were carried out on the leaves and toxicity tests on *Artemia salina*. The results revealed that the aqueous extract, at concentrations of 5 %, 2.5 %, 1.25 % and 0.625 %, presented hatching inhibition rates of 81.8 %, 70.0 %, 47.8 % and 40.4 %, respectively. Phytochemical analysis confirmed the presence of secondary metabolites in the extract. Furthermore, the toxicity test demonstrated that the *C. blanchetianus* extract was non-toxic to *Artemia salina* at all concentrations tested. Consequently, it can be concluded that the aqueous extract had inhibitory effects on egg hatching due to the presence of several secondary metabolites, thus increasing its potential as an anthelmintic treatment against gastrointestinal parasites in sheep.

Keywords: sheep farming; small ruminants; parasites and phytotherapy.

¹ Universidade Federal Rural do Semi-Árido. Mossoró/RN, Brazil. <https://orcid.org/0000-0002-2878-7883>

² Universidade Federal Rural do Semi-Árido. Mossoró/RN, Brazil. <https://orcid.org/0000-0001-6976-754X>

³ Universidade Federal Rural do Semi-Árido. Mossoró/RN, Brazil. <https://orcid.org/0000-0002-2879-6333>

⁴ Universidade Federal Rural do Semi-Árido. Mossoró/RN, Brazil. <https://orcid.org/0000-0003-4012-0659>

⁵ Universidade Federal Rural do Semi-Árido. Mossoró/RN, Brazil. <https://orcid.org/0000-0002-4984-2298>

⁶ Universidade Federal Rural do Semi-Árido. Mossoró/RN, Brazil. <https://orcid.org/0000-0003-1578-3733>

⁷ Universidade Federal Rural do Semi-Árido. Mossoró/RN, Brazil. <https://orcid.org/0000-0002-1884-6042>

⁸ Universidade Federal Rural do Semi-Árido. Mossoró/RN, Brazil. <https://orcid.org/0000-0002-1039-5187>

INTRODUCTION

In Brazil, sheep farming is regarded as one of the primary economic activities, providing income and sustenance for breeders while also serving as a profitable venture and contributing to the food supply of farming families.¹ The expansion of sheep farming across Brazil, particularly in the Northeast region, which accounts for 70.6% of the country's sheep production,² has led to a diversification in commercialization. Previously centered around wool and skin production, the industry has now expanded to include the marketing of meat, milk, and related products.

However, the presence of gastrointestinal endoparasites in sheep herds can hinder their development, leading to weight loss, anemia, and a decline in productivity and reproductive potential. Poor sanitary management practices further contribute to the development of parasitic resistance.³ Consequently, sheep affected by gastrointestinal infections exhibit symptoms that render them unproductive.⁴

Chemical drugs are widely utilized for the treatment of parasites,⁵ but their excessive and improper use contributes to the emergence of parasitic resistance. This, in turn, leads to increased expenses for maintaining the animals, elevated mortality rates, and decreased herd productivity.⁶ Consequently, producers face significant economic losses.⁷

Therefore, phytotherapy has been embraced as a means of controlling diseases caused by gastrointestinal parasites. Various formulations,⁸ including extracts and essential oils, which contain bioactive substances, have been approved for use.⁹ Since these compounds are derived from natural sources, they often possess low toxicity,¹⁰ allowing for their utilization in order to reduce the presence of chemical contaminants in the environment.¹¹

Vegetable-derived compounds have demonstrated efficacy in controlling microorganisms. Medicinal plants, known and validated by popular usage, have exhibited antibacterial, antifungal, ectoparasitic, and anthelmintic properties.¹²

In this context, the species *Croton blanchetianus*, commonly known as the "quince tree," deserves particular attention. It belongs to the Euphorbiaceae family.¹³ This plant species is endemic to Brazil, with a greater presence in the Northeast region.¹⁴ This genus is also known for having a wide production of secondary metabolites, therefore it has been studied for scientific proof of its biological activities.¹⁵ Leaves and bark of this plant are still used today for therapeutic purposes; in folk medicine, quince has traditionally been used to treat problems such as swelling, uterine bleeding, hemoptysis, stomach pain, vomiting and diarrhea.¹⁶ Due to its remarkable potential, quince has been the subject of frequent studies focusing on the biological activity of its essential oils and extracts.¹⁷ However, it is worth highlighting the significant lack of research on the potential of *C. blanchetianus* against gastrointestinal parasites in sheep.

Hence, this study aimed to assess the ovicidal activity and toxicity of the aqueous extract of *C. blanchetianus*, as well as analyze the phytochemical composition of its leaves. The ultimate goal was to establish a viable and effective strategy for the control of gastrointestinal nematodes, benefiting both the environment and society.

MATERIAL AND METHOD

Ethical and Legal Procedures

The research followed all ethical and legal precepts with approval from the Ethics Committee on the Use of Animals n0 14/2021 and registration in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge n0 A137FA3. The plant sample was deposited in the

Herbarium Dárdano de Andrade-Lima, at the Center for Biological Sciences and Health, Universidade Federal Rural do Semi-Árido – Ufersa with exsiccate nº 15132, proving the described species.

Description of the Study Area

The study was conducted in the city of Mossoró located in the western region of the state of Rio Grande do Norte, inserted in the Northeast, with a territorial dimension of 2,099,334 Km². The municipality is located between the geographic coordinates 5º 11' 15" South latitude and 37º 20' 39" West longitude and at an average altitude of 16 meters above sea level. The predominant climate is semi-arid, with two climatic phases: a dry one between June and January, and a rainy one, from February to May. The relative humidity of the air is around 70% and the average annual temperature is 27.4 °C.

Obtaining Plant Material

The leaves of *C. blanchetianus* were collected in the city of Mossoró-Rio Grande do Norte in the morning, then taken to the Laboratory of Biotechnology Applied to Infectious-Parasitic Diseases of the Universidade Federal Rural do Semi-Árido (Ufersa), where the production of the aqueous extract.

Obtaining the Aqueous Extract from the Leaves of *Croton blanchetianus*

The leaves were cleaned and dried at room temperature. After eight days of drying, they were crushed and sieved until obtaining a fine powder that was easy to dissolve. From the vegetable flour obtained, it was subjected to solubilization in a proportion of 5% (weight/volume), where 30 grams of the vegetable were diluted in 600 mL of distilled water. The mixture was left under constant magnetic stirring for 16 hours at 20°C. After the extraction time, the material was filtered through gauze and subjected to centrifugation (8000 rpm, 20 min at 4 °C) to remove insoluble material. The supernatant material was called crude extract (EB). Finally, dilutions in distilled water were prepared from the crude extract, at concentrations of 2.5% and 1.25%, 0.625% .¹⁸

Phytochemical Determination of the Aqueous Extract

The aqueous extract of *C. blanchetianus* leaves was submitted to phytochemical characterization.¹⁹ Metabolic classes were identified from observations of precipitation, colorimetric and fluorescence reactions, indicating their presence or absence. Reactions were carried out to identify coumarins (Fluorescence Test), phenols (Ferric Chloride Reaction), flavonoids (Cyanidin or Shinoda Reaction), anthraquinones (Borträger), steroid nuclei (Liebermann-Burchard Test), triterpene nuclei (Test Salkowski test), saponins (Foam test – vigorous stirring), condensed tannins and hydrolysable tannins (Stiasny test), free tannins (Gelatin precipitation test).

Collection of Fecal Samples

The animals were randomly selected, regardless of breed or sex, belonging to the herds that presented the end of the residual period of 90 days without treatment with synthetic chemicals.²⁰ After the selection of the animals, fecal samples were collected directly from the rectal ampoula, to carry out the counting of eggs per gram of feces (OPG) by weighing 4 g of feces according to the McMaster technique, where fecal samples were separated with OPG equal to or more than 2000 eggs.²¹

Egg Hatch Recovery and Test (EHT)

The recovery of nematode eggs was carried out according to the methodology of Hubert and Kerboeuf,²² with fecal samples diluted in water and passed through a sequence of sieves containing a weight of 0.15; 0.10; 0.036 and 0.02 mm for egg retention. Subsequently, a centrifugation (4,000 rpm;

5 minutes) of the recovered liquid was performed. The sediment from the first centrifugation was resuspended in concentrated saline solution and subjected to a second centrifugation (4,000 rpm; 5 minutes) to obtain the supernatant containing the eggs.

The egg hatch test (EHT) was performed according to the Coles methodology;²³ an average of 100 eggs/well were added in 24-well plates, using five wells for each treatment. The negative control containing eggs incubated in aqueous solution; the positive control containing eggs submitted to chemical treatment with Thiabendazole (32 µl/mL); the experimental group containing the eggs incubated in the crude aqueous extract with concentrations of 5%, 2.5%, 1.25% and 0.625%.

The 24-well plates were incubated in BOD (Biochemical Oxygen Demand) for 48 hours at a temperature of 27 °C and controlled humidity, with subsequent addition of Lugol and counting eggs and larvae in each well, using an inverted microscope.

Toxicity of the Aqueous Extract in *Artemia salina*

The experimental assay to evaluate the toxicity of the essential oil from *C. blanchetianus* was conducted using 24-well plates.²⁴ *Artemia salina* cysts were hatched in filtered saline solution with a concentration of 40 g/L (water with sea salt) and a pH between 8-9. For each liter of water, 100 mg of *A. salina* cysts were added. The hatching process took place at room temperature, under 100 W illumination, with constant aeration, for 48 hours. Ten hatched nauplii were then separated and transferred to 24-well plates containing 100 µl of *A. salina* culture solution and 400 µl of essential oil concentrations from *C. blanchetianus* leaves (10%, 5%, 2.5%, 1.25%). The bioassay was performed in triplicate, with the plant extract tested three times, resulting in a total of nine assays per concentration. The negative control was conducted by adding the culture solution to the wells. Subsequently, the plates were incubated for 24 hours. After the specified time period, the brine shrimps were examined, and the number of live and dead nauplii was recorded. Nauplii showing movement when observed near the light source were considered alive. Tests in which the control group exhibited mortality rates of less than or equal to 10% of the population were considered valid.

Statistical Analysis

The data obtained was organized and recorded in an Excel spreadsheet. The hatching percentage of eggs for each experimental group was calculated using the formula: $(\text{number of eggs} / (\text{number of eggs} + \text{number of L1})) \times 100$. The Shapiro-Wilk test was applied to assess the normality of the data, and significant differences between the analyzed groups were examined using the Kruskal-Wallis test, followed by the Bonferroni test. A significance level of $p < 0.05$ was adopted for the tests. The 50% lethal concentration (LC50) in the *A. salina* toxicity assay will be determined through non-linear regression analysis using the R 4.1.3 software for data analysis.

RESULTS

Inhibition of Egg Hatching

The results of the Egg Hatch Test (EHT) using the aqueous extract of *C. blanchetianus* with concentrations of 5 %, 2.5 %, 1.25 % and 0.625 %. The positive control using the chemical drug Thiabendazole (32 µl/mL) inhibited hatching by 74.4 %, and the negative control (0.15 M NaCl) showed no significant inhibition in relation to egg hatching (10%). The inhibition rates of the aqueous extract of *C. blanchetianus* were higher at concentrations of 5 % (82 %) and 2.5 % (70 %), which, on the other hand, were not statistically different from what we observed in the positive control. However, the results shown for the concentrations of 1.25 % and 0.625 % did not present a statistically significant difference when compared to the results obtained for the negative control (Figure 1).

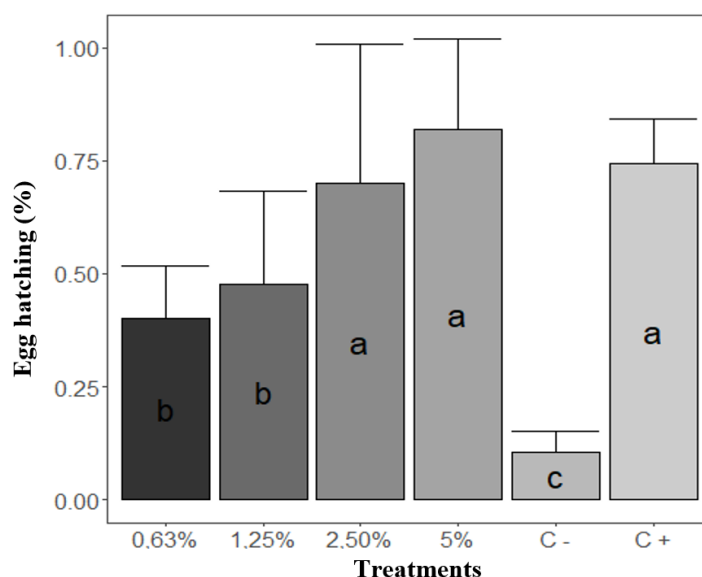


Figure 1. Effect of aqueous extract of *C. blanchetianus* leaves on the hatching of sheep nematode eggs according to concentrations of 5%, 2.5%, 1.25% and 0.625%. *Mean values followed by the same letter do not differ statistically from each other. (Kruskal-Wallis, $p \leq 0.05$).

C- : negative control C+ : positive control

Source: Prepared by the authors.

Phytochemical Analysis

As for the analysis of the chemical composition of the aqueous extract of *C. blanchetianus* leaves, the presence of phenols (Ferric Chloride Reaction), flavonoids (Cyanidin or Shinoda Reaction), steroid nuclei (Liebermann-Burchard Test), saponins (Foam Test – vigorous shaking), alkaloids (characterization reaction – precipitation) condensed tannins and hydrolyzable tannins (Stiasny Test), free tannins (Gelatin Precipitation Test).

Acute Toxicity

Results of the toxicity test with *A. salina*. The aqueous extract of *C. blanchetianus* leaves showed an lethal concentration LC_{50} of 350,907 $\mu\text{L/mL}$ and an R^2 of 0.02, demonstrating that the concentrations tested did not present toxicity in the bioindicator used (Figure 2).

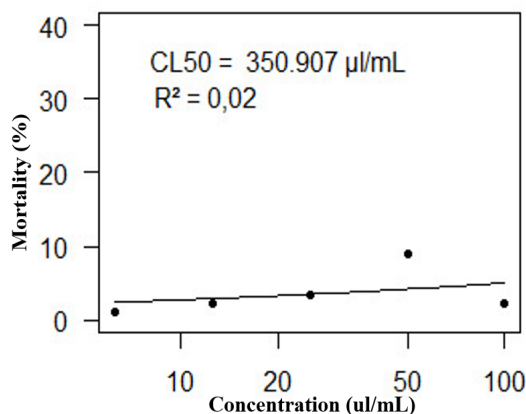


Figure 2 – Representation of the toxicity test of the concentrations used of the aqueous extract of the leaves of *C. blanchetianus* on *A. salina*.

Source: Prepared by the authors.

DISCUSSION

The aqueous extract of *C. blanchetianus* demonstrated effectiveness in inhibiting egg hatching at concentrations of 5% and 2.5%. However, the percentage of inhibition observed in the positive control was 74.40%, indicating potential evidence of anthelmintic resistance.²⁵

According to Coles,²³ an OPG (eggs per gram) count below 90% is indicative of suspected parasitic resistance. Parasites possess various biological and genetic characteristics that contribute to the development of resistance against chemical drugs. Factors such as a high reproduction rate, short life cycles, and a large population size provide helminths with genetic diversity.^{6, 26}

Considering the challenge of parasitic resistance, plants used for medicinal purposes can be seen as an economically viable alternative for the control and treatment of gastrointestinal parasites, in addition to demonstrating the potential as an anthelmintic in small ruminants, without dependence on the preparation process.²⁷ Thus, he states that anthelmintic activity has been reported from plant extracts used in gastrointestinal parasites.²⁸ Studies carried out with extracts of plants such as *Carica papaya* and *Musa paradisiaca* and larvae of parasites such as *Haemonchus contortus*.²⁸ Research developed with essential oil of *Cuminum cyminum* proved the effectiveness in the hatching of eggs and in the larval development of parasites.²⁹ Tests also with aqueous extract of leaves of the *Solanum lycocarpum* plant demonstrated the efficiency of its use through the modified larval hatchability technique, enabling a reduction in the hatching of gastrointestinal parasite eggs.³⁰

The ovicidal activity of the extract may be related to the constituents found in the leaves of *C. blanchetianus*, called secondary metabolites: phenols, alkaloids, saponins, flavonoids, steroid terpenes, general tannins and condensed tannins. The secondary metabolites present in plants with anthelmintic property have shown promising results in the fight against gastrointestinal nematodes.³¹

Phenols are commonly found in plants and are known to accumulate in all parts of plants, such as: roots, stems, leaves, fruits and flowers.³² Due to their chemical diversity, phenols perform several functions such as coupling with digestive proteins to inactivate important enzymes in this process.³³ According to a study carried out, phenols interfere with the parasite's energy production mechanism, as it uncouples oxidative phosphorylation, compromising proteins that remain on the surface of nematode cells, causing the parasite's death.³⁴

Tannins and flavonoids are other phenolic compounds present in the extract of *C. blanchetianus* leaves. Tannins are considered natural sources and are widely distributed in stems, leaves and inflorescences of plants, being generally divided into condensed and water-soluble tannins.^{35,36} They have antiparasitic action that may be related to their ability to interact and form complexes with the proteins of the parasites, impairing the absorption of nutrients, directly affecting motility and reproduction and larval development and egg hatching.

Flavonoids have shown positive effects related to the health of animals, as they have antioxidant activity and the ability to interact with enzymes present in parasites, causing changes in their energetic pathways of essential metabolic substances for energy absorption.³⁷

Alkaloids form the second largest family of secondary metabolites and can be found in various animals, microorganisms and plants.³⁰ As a biological activity, alkaloids can act in the nervous system, as a neurotransmitter, acting as a sedative, analgesic and muscle relaxant, causing loss of motility and stretching of the parasite, inhibiting its movement.³⁸

Terpenes are present in products of plant origin such as extracts and essential oils, so they act by attracting pollinators. They also have insecticidal, antimicrobial, anti-inflammatory, among others.³⁹

Saponin was also identified in the aqueous extract, which is an important secondary metabolite of plants, considered a natural compound with anthelmintic action. The biological effects of saponin

have been related to its ability to form complexes with the membrane of the parasite, altering its permeability and promoting the entry and exit of molecules, resulting in the formation of vacuoles in the cytoplasm of cells, causing the disintegration of the worm's envelope, impairing its development or causing its death.

The toxicological effect of the concentrations of the aqueous extract of *C. blanchetianus* leaves was determined through the Average Lethal Concentration (LC50). The relationship between toxicity and average lethal concentration presented by plant extracts on *Artemia salina* is from the LC50 values, above 1000 µg/mL are considered non-toxic, LC50 greater than 500 µg/mL presents a low toxicity, LC50 is moderate with values between 100 to 500 µg/mL and toxic lower than 100 µg/mL. Thus, the toxicity test result shows the LC50 value equal to 350,907 µg/mL. Thus, we can see a low level of effect on mortality with the concentrations tested, with a maximum mortality of 40%. In this context, the aqueous extract of *C. blanchetianus* leaves was considered non-toxic in the *in vitro* test, as it presented an LC50 value greater than 1000 µg/mL.⁴⁰ These results may be due to the diversity of aspects among individuals of the same species, known as polymorphism, such as temperature, humidity, solar radiation and genetic variability.⁶

Therefore, this study revealed that *C. blanchetianus* is a species that presents interesting and beneficial characteristics when it comes to the formulation of plant-based products against gastrointestinal nematodes. The aqueous extract showed an *in vitro* ovicidal effect at all concentrations tested and did not show toxicity. In addition, it is a great alternative to replace conventional methods, as it is a viable strategy for use due to its wide availability in semiarid regions, low cultivation cost and ease of preparation. Its drought resistance and low fertilization requirements reduce operational costs, while its easy adaptation makes it ideal for sheep feeding. Therefore, *C. blanchetianus* has several promising secondary metabolites with proven efficacy.

CONCLUSION

The aqueous extract of *C. blanchetianus* leaves was shown to be able to inhibit the hatching of eggs of gastrointestinal parasites in sheep. Making the plant an alternative source for the control of gastrointestinal helminths. The use of plant-based medicines can reduce cases related to parasite resistance, as well as environmental impacts. Thus, we affirm that the results obtained offer a promising basis for future *in vivo* research, which is relevant to validate the practical applicability of herbal medicines in the parasite management of sheep.

REFERENCES

- ¹ Agne CL, Porto AR, Cavalheiro M. Perfil dos consumidores de carne ovina no município de Cachoeira do Sul – RS, Brasil. Rev. Grifos. 2021 June 14;30(53):72-93. DOI: <https://doi.org/10.22295/grifos.v30i53.5627>
- ² IBGE. Instituto Brasileiro de Geografia e Estatística. Produção Agropecuária. 2022 [cited 2022 May 30]. Available from: <https://www.ibge.gov.br/explica/producao-agropecuaria/ovino/br>
- ³ Naeem M, Iqbal Z, Roohi N. Ovine haemonchosis: a review. Trop. Anim. Health Prod. 2020 Nov. 20;53(19):1-11. DOI: <https://doi.org/10.1007/s11250-020-02439-8>
- ⁴ Idris OA, Wintola OA, Afolayan AJ. Helminthiasis; prevalence, transmission, host-parasite interactions, resistance to common synthetic drugs and treatment. Heliyon. 2019 Jan.;5(1):e01161. DOI: <https://doi.org/10.1016/j.heliyon.2019.e01161>
- ⁵ Jiao Y, Preston S, Garcia-Bustos JF, Baell JB, Ventura S, Le T, et al. Tetrahydroquinoxalines induce a lethal evisceration phenotype in *Haemonchus contortus* *in vitro*. Int Journal Parasitol: Drugs and Drug Resistance. 2019 Apr. 1;9:59-71. DOI: <https://doi.org/10.1016/j.ijpddr.2018.12.007>

- ⁶ Castro EMS, Souza EAR, Dantas ACS, da Silva IWG, de Araújo MM, de Azevedo SS, et al. Resistência parasitária de nematódeos gastrintestinais de caprinos criados em região semiárida de Pernambuco, nordeste do Brasil. *Vet. Zootec, Botucatu*. 2021 Mar. 29;28(1):1-12. DOI: <https://doi.org/10.35172/rvz.2021.v28.523>
- ⁷ Aguiar KPA, Ramos LM, de Oliveira RAP, Guimarães CRR. Manejo sanitário de caprinos e ovinos. *Rev Multidisc Nordeste Min*. 2024;8(1). DOI: <https://doi.org/10.35172/rvz.2021.v28.523>
- ⁸ Cruz IR de L, de Andrade JF, Viana ALA, Pereira MF, Sales LB de O, do Nascimento AJB, et al. Controle fitoterápico e fúngico de parasitoses na criação de ruminantes. *Braz J Dev [Internet]*. 2023 May 9 [cited 2024 Sept. 10];9(5). DOI:10.34117/bjdv9n5-069
- ⁹ Ferreira ET, dos Santos ES, Monteiro JS, Gomes M do SM, Menezes RA de O, de Souza MJC. A utilização de plantas medicinais e fitoterápicos: uma revisão integrativa sobre a atuação do enfermeiro. The use of medicinal and phytotherapy plants: an integrational review on the nurses performance. *Braz J Health Rev*. 2019 Feb. 27;2(3):1511-1523.
- ¹⁰ Lima PR, Apdini T, Freire AS, Santana AS, Moura LML, Nascimento JCS, et al. Dietary supplementation with tannin and soybean oil on intake, digestibility, feeding behavior, ruminal protozoa and methane emission in sheep. *Anim. Feed Sci Technol*. 2019 Mar.;249:10-7. DOI: <https://doi.org/10.1016/j.anifeedsci.2019.01.017>
- ¹¹ Stucki K, Cero MD, Vogl CR, Ivemeyer S, Meier B, Maeschli A, et al. Ethnoveterinary contemporary knowledge of farmers in pre-alpine and alpine regions of the Swiss cantons of Bern and Lucerne compared to ancient and recent literature – Is there a tradition? *J. Ethnopharmacol*. 2019 Apr.;234:225-244. DOI: <https://doi.org/10.1016/j.jep.2018.12.022>
- ¹² Silva CR, da Silva NCS, Costa-Júnior LM, Nunes GS. Avanços no desenvolvimento de tecnologias utilizando óleos essenciais para controle de parasitos de pequenos ruminantes. *Rev Gest Inov Tecnol*. 2019 May;9(2):4966-4976. DOI: 10.7198/geintec.v9i2.1265
- ¹³ Nunes AKA, Malveira EA, Andrade AL, da Silva WMB, de Moraes SM, dos Santos HS, et al. Chemical Composition Determination and Evaluation of the Antimicrobial Activity of Essential Oil from *Croton blanchetianus* (Euphorbiaceae) against Clinically Relevant Bacteria. *Chem Biodivers*. 2022 Dec 21;20(1):e202200777. DOI: <https://doi.org/10.1002/cbdv.202200777>
- ¹⁴ Gomes AP de S, de Sales MF, de Melo AL. Novidades taxonômicas em *Croton* sect. *Argyroglossum* Baill. e *C. sect. Lasiogyne* Klotzsch (Crotonoideae-Euphorbiaceae). *Acta Bot Bras*. 2010 Dec. 1;24(4):905-908. DOI: <https://doi.org/10.1590/S0102-33062010000400004>
- ¹⁵ Okokon JE, Nwafor PA. Antiinflammatory, analgesic and antipyretic activities of ethanolic root extract of *Croton zambesicus*. *Pak J Pharm Sci*. 2010;23(4):385-392.
- ¹⁶ Matos FJ de A, Machado MIL, Craveiro AA, Alencar JW, Barbosa JM, da Cunha EVL, et al. Essential Oil of *Mentha villosa* Huds. from Northeastern Brazil. *J Essent Oil Res*. 1999 Jan.;11(1):41-44. DOI: <https://doi.org/10.1080/10412905.1999.9701066>
- ¹⁷ Pinho-da-Silva L, Mendes-Maia PV, Garcia TM do N, Cruz JS, de Moraes SM, Coelho-de-Souza AN, et al. *Croton sonderianus* essential oil samples distinctly affect rat airway smooth muscle. *Phytomedicine*. 2010 Aug. 1;17(10):721-725. DOI: <https://doi.org/10.1016/j.phymed.2010.01.015>
- ¹⁸ Nelson DL, Cox MM. Princípios de bioquímica de Lehninger. Porto Alegre: Artmed; 2014.
- ¹⁹ Francisco M. Introdução à fitoquímica experimental. Fortaleza: Edições UFC; 2009.
- ²⁰ Niciura SCM, Veríssimo CJ, Molento MB, editors. Determinação da eficácia anti-helmíntica em rebanhos ovinos: metodologia de colheita de amostras e de informações de manejo zoossanitário [Internet]. São Carlos: Embrapa Pecuária Sudeste; 2009 Nov. [cited 2021 Jan. 15]. Available from: <https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/578348/1/PROCIDOC91SCMN2009.00152.pdf>
- ²¹ Gordon HM, Whitlock HV. A New Technique for Counting Nematode Eggs in Sheep Faeces; 1939.
- ²² Hubert J, Kerboeuf D. A microlarval development assay for the detection of anthelmintic resistance in sheep nematodes. *Vet Rec*. 1992 May 16;130(20):442-446. DOI: <https://doi.org/10.1136/vr.130.20.442>
- ²³ Coles GC, Jackson F, Pomroy WE, Prichard RK, Samson-Himmelstjerna G von, Silvestre A, et al. The detection of anthelmintic resistance in nematodes of veterinary importance. *Vet Parasitol [Internet]*. 2006 Mar. 31;136(3-4):167-185. DOI: <https://doi.org/10.1016/j.vetpar.2005.11.019>
- ²⁴ Meyer B, Ferrigni N, Putnam J, Jacobsen L, Nichols D, McLaughlin J. Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. *Planta Med*. 1982 May;45(05):31-4. DOI: 10.1055/s-2007-971236
- ²⁵ Osório TM, Menezes L de M, Rosa KB, Escobar RF, Lencina RM, Maydana G de M, et al. Resistência anti-helmíntica em nematódeos gastrintestinais na ovinocultura: uma revisão. *Braz J Dev*. 2020;6(11):89194-89205. DOI: <https://doi.org/10.34117/bjdv6n11-368>

- ²⁶ Kaplan RM. Biology, Epidemiology, Diagnosis, and Management of Anthelmintic Resistance in Gastrointestinal Nematodes of Livestock. *Vet Clin North Am Food Anim Pract.* 2020 Mar.;36(1):17-30. DOI: <https://doi.org/10.1016/j.cvfa.2019.12.001>
- ²⁷ Vanessa Daniele Mottin, Mottin VD, da Cruz JF, Neto MRT, Marisco G, Figueredo JS, et al. Efficacy, toxicity, and lethality of plants with potential anthelmintic activity in small ruminants in Brazil. *Rev Bras Saúde Prod Anim.* 2019 Jan. 19;20:01-23. DOI: <https://doi.org/10.1590/S1519-9940200232019>
- ²⁸ Krychak-Furtado S, Negrelle RB, Miguel OG, Zaniolo SR, Kapronezai J, Ramos SJ, et al. Efeito de *Carica papaya* L. (Caricaceae) e *Musa paradisiaca* Linn. (Musaceae) sobre o desenvolvimento de ovos de nematódeos gastrintestinais de ovinos. *Arq Inst Biol.* 2005 Apr.;72(2):193-199. DOI: <https://doi.org/10.1590/1808-1657v72p1932005>
- ²⁹ Castro, L. M., Pinto, N. B., de Moura, M. Q., Freitag, R. A., et al. In vitro activity of the essential oil of *Cuminum cyminum* against *Haemonchus contortus* of sheep. *Braz J Dev.* 2021;7(5):44079-44091. DOI: 10.34117/bjdv.v7i5.29188
- ³⁰ Oliveira, A. G. N., Capello, A. B. T., da Silva Sunada, N., & Fraga, T. L. Tecnologia para produção de extrato aquoso de folhas da planta *Solanum lycocarpum* para avaliação da eficiência anti-helmíntica: validação da técnica de inibição da eclodibilidade larval de helmintos de ovinos. *Vet e Zootec.* 2022;29:1-7. DOI: <https://doi.org/10.35172/rvz.2022.v29.675>
- ³¹ Santos TFB dos, Souza AM de, Rodrigues WB, Barata J da S, Oliveira ACP de, Amorim SL de. Atividade anti-helmíntica do extrato etanólico das folhas da *Arrabidaea chica* sobre ovos e larvas de nematódeos gastrintestinais de ovinos. *Sci Nat.* 2021;3(1):172-178. DOI: <https://doi.org/10.29327/269504.3.1-16>
- ³² Liu Y, Qi Y, Chen X, He H, Liu Z, Zhang Z, et al. Phenolic compounds and antioxidant activity in red- and in green-fleshed kiwifruits. *Food Res Int [Internet].* 2019 Feb. 1;116:291-301. DOI: <https://doi.org/10.1016/j.foodres.2018.08.038>
- ³³ Marcucci MC, Salatino A, de Magalhães Oliveira LFA, Gonçalves CP. Metodologias acessíveis para a quantificação de flavonoides e fenóis totais em própolis. *Rev Virtual Quím.* 2021;13(1):1-13.
- ³⁴ John J, Mehta A, Shukla S, Mehta P. A report on anthelmintic activity of *Cassia tora* leaves. *Songklanakarin J Sci Technol.* 2009 Aug. 1;31(3).
- ³⁵ Pineda CO, Temesgen TT, Robertson LJ. Multiplex Quantitative PCR Analysis of Strawberries from Bogotá, Colombia, for Contamination with Three Parasites. *J Food Prot.* 2020 May 18;83(10):1679-1684. DOI: <https://doi.org/10.4315/JFP-20-121>
- ³⁶ Olmedo-Juárez A, Zarza-Albarran MA, Rojo-Rubio R, Zamilpa A, González-Cortazar M, Mondragón-Ancelmo J, et al. *Acacia farnesiana* pods (plant: Fabaceae) possesses anti-parasitic compounds against *Haemonchus contortus* in female lambs. *Exp Parasitol.* 2020 Nov. 1;218:107980. DOI: <https://doi.org/10.1016/j.exppara.2020.107980>
- ³⁷ Mercês ZC, Santos JCDM. Compostos bioativos – flavonoides. 2023 Mar. 6 [cited 2024 Sept. 12];1-12. Available from: <https://arqcientificosimmes.emnuvens.com.br/abi/article/view/548>
- ³⁸ Silva AI, Sá-Filho G, Oliveira L, Guzen F, Cavalcanti J, Cavalcante J. Perfil fitoquímico de extratos etanólicos e metanólicos do *Croton blanchetianus*. *Rev Bras Multidiscipl.* 2021 Apr. 22;24(1):134-142. DOI: <https://doi.org/10.25061/2527-2675/ReBraM/2021.v24i1.1057>
- ³⁹ Teshome E, Forsido SF, Rupasinghe HPV, Keyata EO. Potentials of Natural Preservatives to Enhance Food Safety and Shelf Life: A Review. *Sci World J.* 2022 Sept. 23;2022(1):9901018. DOI: <https://doi.org/10.1155/2022/9901018>
- ⁴⁰ Martins ACR, da Costa JKN, Herbert A, Farias FRS, Rezende M, Junior VAK, et al. Avaliação da toxicidade das tinturas de aroeira e de romã através do bioensaio com *Artemia salina*. *Res Soc Dev.* 2021 Mar. 24;10(3):e52010313751-e52010313751.

Submitted: September 18, 2024

Accepted: February 17, 2025

Published: July 25, 2025

Authors' contributions	
Yandra Thaís Rocha da Mota:	Conception and design of the study and preparation of the manuscript.
Ana Karolinne de Alencar França:	Literature review and preparation of the manuscript.
Marcus Vinicius Gomes Dantas:	Literature review and data analysis and interpretation.
Renata Cristina Borges da Silva Macedo:	Data analysis and interpretation and data acquisition
João Inácio Lopes Batista:	Data acquisition.
Karoline Mikaelle de Paiva Soares:	Conception and design of the study.
Michele Dalvina Correia da Silva:	Conception and design of the study and intellectual review of the manuscript.
Ana Carla Diógenes Suassuna Bezerra:	Conception and design of the study and intellectual review of the manuscript.
All the authors approved the final version of the text.	
Conflict of interest:	There is no conflict of interest.
Financing:	No financing.
Corresponding author:	Yandra Thaís Rocha da Mota Universidade Federal Rural do Semi-Árido R. Francisco Mota, 572 – Pres. Costa e Silva, Mossoró/RN, Brazil. Zip code 59625-900 yandra.tais@gmail.com
Editor:	Matias Nunes Frizzo. Ph.D
Editor-in-chief:	Adriane Cristina Bernat Kolankiewicz. Ph.D

This is an open-access article distributed under the terms of the Creative Commons license.

