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In-vitro effect of the methanolic extract of *Morinda citrifolia* against the life cycle of *Dermatobia hominis*

Efecto *in-vitro* del extracto metanólico de *Morinda citrifolia* sobre el ciclo de vida de *Dermatobia hominis*

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ABSTRACT

Background: bovine cutaneous dermatobiosis or furuncular myiasis caused by *Dermatobia hominis* is a parasitosis that mainly affects bovines in the tropics and represents a particular interest in public health as zoonosis. Its control is based on ivermectins, which have long withdrawal times, affecting the productive dynamics within dairy cattle herds. **Objective:** to assess the *in-vitro* effect of the methanolic extract of the *M. citrifolia* ripe fruit against the life cycle of *D. hominis*. **Methods:** *D. hominis* larvae were taken directly from naturally parasitized bovine skins. These larvae were exposed by immersion to different concentrations of the methanolic extract of *M. citrifolia* (10, 50, 100, 200, 300, 400, 460 mg/mL) diluted in distilled water. Ivermectin 1% was used as a positive control, and distilled water as a negative control. Subsequently, the larvicidal activity was evaluated in the first 48 hours post-immersion (PI), the pupicidal activity within 10 to 23 days PI, and the inhibition of the imagos emergence as well as their anatomical alterations, were evaluated within 24 to 35 days PI; recreating the pupal development and their hatching in the soil under controlled laboratory conditions. CL_{50} and CL_{90} for the larvae phase were estimated through Probit regression analysis. **Results:** *M. citrofolia* concentrations of 400 and 460 mg/mL had a significant ($p<0.05$) larvicidal effect of 40% (95% CI 34.7 - 43.9) and 60% (95% CI 56.8 - 67.3), respectively. The pupicidal effect on the surviving larvae was significant ($p<0.05$) at 300, 400, and 460 mg/mL: 40% (95% CI 37.9 - 42.3), 60% (95% CI 55.7 - 65.9) and 70% (CI 95% 67.1 – 76.7), respectively. The inhibition of the emergence of imagos was significant ($p<0.05$) 50% (95% CI 42.3 - 57.8) in all concentrations equal to or greater than 200 mg/mL. Finally, 20% (95% CI 12.6 - 29.3) of the emerging imagos at 460 mg/mL presented morphoanatomy alterations ($p<0.05$). The LC_{50} and LC_{90} estimated (larval phase) were 22.36 mg/mL (95%CI 15.06-33.19) and 245.08 mg/mL (95%CI 165.10-363.82), respectively. **Conclusions:** The methanolic extract of *M. citrifolia* was effective as larvicide, altering the pupation and the emergence of imagos of *D. hominis*. In addition, it modified the imagos morphoanatomy; interesting results to promote *in-situ* and other bioguided fractionation studies of this extract in different *D. hominis* stages; being *M. citrifolia* a plant species widely adapted to the conditions of the Meta department, Colombia.

Keywords: cattle, myiasis, parasite control, phytotherapy.

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RESUMEN

Antecedentes: Dermatobiosis cutánea bovina o miasis furuncular causada por *Dermatobia hominis*, es una parasitosis que afecta principalmente bovinos de los trópicos y representa interés particular en salud pública como zoonosis. Su control se sustenta en el uso de ivermectinas, las cuales tienen prolongados tiempos de retiro, afectando la dinámica productiva dentro de los hatos bovinos lecheros. **Objetivo:** Evaluar el efecto *in-vitro* del extracto metanólico del fruto maduro de *Morinda citrifolia* en el ciclo de vida de *D. hominis*. **Métodos:** Se tomaron larvas de *D. hominis* directamente de pieles de bovinos parasitados naturalmente. Estas larvas fueron expuestas por inmersión a diferentes concentraciones del extracto metanólico de *M. citrifolia* (10, 50, 100, 200, 300, 400, 460 mg/mL) diluido en agua destilada. Se utilizó ivermectina 1% como control positivo, y agua destilada como control negativo. Posteriormente, se evaluó la actividad larvicida en las primeras 48 horas post-inmersión (PI), pupicida dentro de los 10 a 23 días PI, inhibición en la emergencia y alteraciones anatómicas de imagos, dentro de los días 24 al 35 PI; recreando el desarrollo pupal y su eclosión en el suelo, bajo condiciones controladas de laboratorio. La obtención de CL_{50} y CL_{90} de la fase larval se realizó a través de regresión lineal por el método Probit. **Resultados:** Las concentraciones de *M. citrofolia* de 400 mg/mL y 460 mg/mL tuvieron un efecto larvicida (T1) significativo ($p<0.05$) del 40% (IC 95% 34.7 – 43.9) y 60% (IC 95% 56.8 – 67.3), respectivamente. El efecto pupicida (T2) de las larvas sobrevivientes fue significativo ($p<0.05$) en el orden del 40% (IC 95% 37.9 - 42.3), 60% (IC 95% 55.7 – 65.9) y 70% (IC 95% 67.1 – 76.7) en 300, 400 y 460 mg/mL, respectivamente. La inhibición en la emergencia de imagos fue significativa ($p<0.05$), 50% (IC 95% 42.3 – 57.8) en todas las concentraciones iguales o mayores a 200 mg/mL. Finalmente, el 20% (IC 95% 12.6 – 29.3) de los imagos emergentes en 460 mg/mL, presentaron alteraciones morfoanatómicas ($p<0.05$). Se estimaron CL_{50} y CL_{90} (fase larval) de 22.36 mg/mL (95%IC 15.06-33.19) y 245.08 mg/mL (95%IC 165.10-363.82), respectivamente. **Conclusiones:** El extracto metanólico de *M. citrifolia* fue eficaz larvicida, alterando el empupamiento y la emergencia de imagos de *D. hominis*. Además, modificó la morfoanatomía de los imagos; acción importante para promover otros estudios *in-situ* y de fraccionamiento bioguiado de este extracto en diferentes estadios de *D. hominis;* siendo *M. citrifolia* una especie vegetal ampliamente adaptada a las condiciones del departamento del Meta, Colombia.

Palabras clave: Control de parásito, fitoterapia, ganadería bovina, miasis.

INTRODUCTION

The presence of *Dermatobia hominis* (Linnaeus Jr., 1781), also known as torsalo, american warble fly, or the human botfly; has shown over the years to have a significant economic impact on bovine production systems, calculating annual losses in tropical regions such as Colombia and Venezuela in more than 260 million dollars (1) and in Brazil losses of 380 million dollars a year (2). Bovine cutaneous dermatobiosis (BCD), also called furuncular myiasis or subcutaneous nodular myiasis, is caused by the larval stages of the fly *Dermatobia hominis* (Diptera: *Cuterebridae*) (3).

D. hominis requires mammals as an intermediate host to complete its life cycle (4). In this process, adult *D. hominis* flies capture phoretic hematophagous Diptera (e.g., an adult female can capture up to 16 vectors, especially *Aedes* sp., *Stomoxys calcitrans*, *Musca domestica*, and *Haematobia irritans*) in which they deposit between 4 and 52 eggs (5) and when these carrier flying arthropods land on a mammal, the eggs react by thermotropism by hatching, thus the larvae penetrate through the skin lesion caused by the proboscis of the phoretic arthropod or by some previous skin trauma, remaining in the cutaneous and subcutaneous tissue of the host from 4 to 14 weeks during its obligate parasitic phase; during this period the larva can grow from 1 to 25 mm long and 0.3 to 10 mm wide (6). Between 33 and 42 days after infestation, the stage 3 larvae (L3) complete their development and begin to leave the host in order to finalize the process of sclerotizing the pupal stage for 30-90 days in the soil. Two or three days later, after an imago (young adult) emerges, begins the reproductive phase, with a maximum lifetime of 9 days (7).

In production animals, dermatobiosis represents significant damage, especially to cattle, causing irreversible perforations in their skins and compromising their quality (8). In production, it is considered damaged due to poor sanitary practices (9). However, this is not the only problematic situation in the host animals of this parasite, the BCD is suffered from inflammation and secondary bacterial infection, fluid secretion, and painful skin lesions for the animal (e.g., abscesses and nodules), causing restlessness and weight loss (10) due to less feed consumption, which leads to a decrease in milk and/or meat production (11). Likewise, as a zoonosis, it is frequently reported (12, 13, 14, 15) as a worrying situation due to the significant increase of cases (oral, auricular, ocular, nasal, but also with gastrointestinal and genitourinary manifestations) (4).

The production of bovine cattle faces significant challenges for its development within which, the sanitary problems associated with the management of ectoparasites demonstrate an important economic cost related to veterinary medical services and treatments, the most common based on macrocyclic lactones (MLs) is beginning to be widely discussed due to the potential risk of resistance by *D. hominis* (16). Conventional treatments with

ivermectin and moxidectin in cattle have been associated with parasite permanence on cattle days after treatment and with doramectin in 90% and 70% of treated animals (17). In addition to these discouraging results, the adverse effects of MLs have been widely documented, especially the negative environmental impact on beneficial organisms associated with manure and soil renewal (18).

M. citrifolia is a promising plant due to its multiple phytotherapeutic activities, such as those reported by Nayak and Mengi (21) in a study that evaluated the immunostimulant activity of the *M. citrifolia* extract, in which, the potentiation of the host defense mechanism was demonstrated by stimulating the phagocytic capacity of neutrophils and generating an increase in the release of Interleukin 6 (IL-6). This could be related to an important response to bacterial infections. Likewise, Trieu *et al.* (22) demonstrated the anti-inflammatory, antioxidant, and healing activity of the *M. citrifolia* extract, identifying active phytoconstituents against *Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus.* In addition, observed the healing-promoting activity with significant wound reduction and histological regeneration in continuous treatment with the *M. citrifolia* extract in mice.

The aim of this work was to assess the *in-vitro* effect of the methanolic extract of the *M. citrifolia* ripe fruit against the life cycle of *D. hominis.* In this way, generate the bases to study through bioguided fractionation, a possible therapeutic alternative for the control of *D. hominis* causing BCD in animals from tropical regions. Despite the lack of research precedents with similar experiences, this work generates great expectations about the possibility of providing the conceptual bases for the future implementation of an alternative treatment for the management of this parasitosis, in order to avoid productive economic losses, to reduce its impact in public health as well as to reduce the environmental damage generated by the use of medicines in production animals.

MATERIALS AND METHODS

Methanolic extract preparation and preliminary phytochemical screening

The collection of 10 kg of *Morinda citrifolia* (noni) ripe fruit was carried out at Agrosavia Research Center - *La Libertad* (Villavicencio, Meta, Colombia), during the rainy season. The plant taxonomic identification was carried out in the Llanos Herbarium at *Universidad de los Llanos.* In order to obtain the methanolic extract of the collected fruit, it was washed, chopped, and dried in a recirculating air oven at 40°C for 72 hours. Then, the dry material was pulverized, obtaining a homogeneous sifting. Subsequently, the continuous percolation technique was carried out until exhaustion, with methanol at 98% (Merck®, Germany). The percolated liquid was filtered and concentrated at 56°C in a rotary evaporator at reduced pressure and was finally dried in a water bath.

The secondary metabolites determination was carried out through the colorimetry technique following the methodology proposed by Sanabria (23). These methods determine secondary metabolite groups, such as alkaloids, coumarins, glucosides, anthraquinones, flavonoids, tannins, steroids, quinones, and cardiotonics.

Obtaining L3 larvae of *Dermatobia hominis*

D. hominis larvae were collected in slaughtered animals at *Friogoriente* abattoir (Villavicencio, Meta, Colombia). 300 L3 larvae were collected following the methodology proposed by Pires (24). Briefly, larvae were recovered by pressing the parasitic nodules from the skins of naturally infested bovines previously sacrificed. Euthanasia protocols of the animal slaughterhouse center were endorsed by the Instituto Nacional de Vigilancia de Medicamentos y Alimentos (INVIMA), according to national regulations regarding animal welfare, which enable the operation of the place. This research was approved by Research Center of the Faculty of Human Sciences and Education, attached to the Research General Direction at Universidad de Los Llanos, through the project "Evaluation of the *in-vitro* larvicidal effect of the methanolic extract of *Morinda citrifolia* (noni) on *Dermatobia* spp, addressed to cattle little producers in the rural zone of Puente Amarillo in Restrepo, Meta, Colombia" according to announcement 01-P-2015, which guarantees the humane treatment of the experimental animals used.

Subsequently, larvae were classified based on morphological keys as indicated by Páez and Villa (25) and Francesconi and Lupi (26). On the other hand, larvae were also weighed, looking for homogeneous L3 larvae around 450 mg, managing to establish a standard maturity of the optimal larval stage for the test.

Larval immersion test, pupation test, and imago emergence

Nine experimental groups of *D. hominis* L3 larvae were randomly formed, each one containing ten larvae organized in Petri dishes. Larvae were immersed for 10 minutes (27) in 10 mL of different concentrations of the methanolic extract of *M. citrifolia* dissolved in distilled water (Group 1: 10 mg/mL, Group 2: 50 mg/mL, Group 3: 100 mg/mL, Group 4: 200 mg/mL, Group 5: 300 mg/mL, Group 6: 400 mg/mL and Group 7: 460 mg/mL); following the study methodology proposed for plant extracts with antiparasitic pharmacological activity proposed by Castro *et al*. (28); in addition to two control groups: positive group with ivermectin at 1% and negative group with distilled water. Three replicates were made by test.

After the larval immersion test, L3 larvae of *D. hominis* from each experimental group were placed in metal mountings of 20 cm in diameter and 30 cm deep, surrounded by a plastic mesh (avoiding the escape of imagos after emergence). Larvae were exposed to the pupation substrate, thus ensuring the following stages of their life cycle: pre-pupation, pupation, and imagos emergence; under controlled laboratory conditions (Relative Humidity of 80-90%, 28°C of Temperature and light/darkness cycles of 12 hours). The substrate was sandy clay loam soil with 15 to 25% clay, more than 55% sand, and less than 25% silt (29, 30).

The assemblies with the substrate were evaluated as follows:

Time 1 - prepupal period (T1): up to 2 days after post-immersion of the L3 larvae; evaluation time of their ability to penetrate the substrate, which should be around 5 cm deep (30). After this time, the larvae that did not penetrate the substrate were reported as dead (larvicidal effect), verified by stereoscopy to prove the absence of movements to the thermal stimulus or CO_2.

Time 2 – pupal period (T2): from day 10 to 23 after being placed on the substrate, evaluation time of the pupation process, specifically the lack of formation of anterior spiracles characteristic of *Dermatobia* spp. pupae (pupicidal effect) (29). This methodology was made under stereoscopy after digging up the pupae in each mounting. After this evaluation, pupae were returned to their substrate at the same depth where were found.

Time 3 –imagos emergence (T3): from day 24 to 35 after exposure to the substrate, during this time, the inhibition of the imagos emergence was evaluated. In addition, particular characteristics were assessed according to the anatomical description proposed by Moya (31), specifically anomalies characterized by the lack of development of the head seen through the stereoscope.

Statistical analysis

The larvicidal capacity of the methanolic extract of *M. citrifolia* found in T1, the alteration in the conformation of the pupal stage reported in T2 (pupicidal effect); the inhibition of the emergency of imagos, and the anatomical malformations reported in T3 were expressed in frequencies, percentages (%) and 95% confidence intervals (95% CI). Since these data have an all-or-nothing behavior, a non-parametric statistical design was applied. The Kruskal-Wallis test was run with a significance level of $p<0.05$. The LC_{50} and LC_{90} were estimated from the larvicidal phase results through the Probit regression model analysis (estimate plus 95% confidence interval). The data were organized and analyzed with OpenStat 4.0, version 7.0 statistical program.

RESULTS

Figures 1A and 1B show the comparative results regarding the frequency of larval mortality and the pupicidal effect of *D. hominis* exposed to different concentrations of the methanolic extract of *M. citrifolia*, as well as the positive and negative controls of the test at T1 and T2, respectively. 40% and 46% ($p<0.05$) concentrations of the *M. citrifolia* extract and the positive control (Ivermectin 1%) ($p<0.01$) showed significant differences with respect to the negative control (distilled water) and G1 (10 mg/mL), both in the prepual period (T1) as in the pupal period (T2), respectively. According to the Probit regression results, the LC_{50} estimated was 22.36 mg/mL (95%CI: 15.06-33.19) and the estimated LC_{90} was 245.08 mg/mL (95%CI: 165.10-363.82). In addition, regarding the morphological differences of the pupal phase, the experimental Group 7 (460 mg/mL) did not present spiracles (Figure 2A).

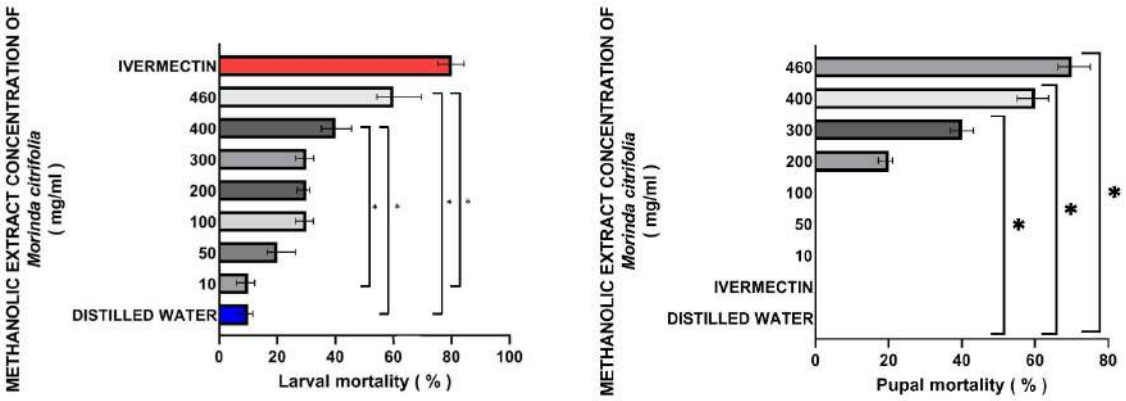
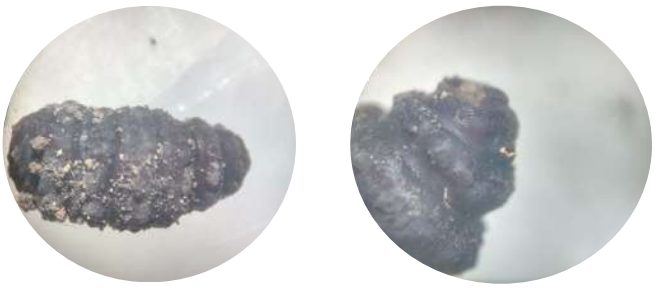
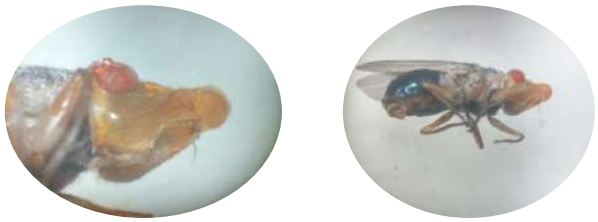


Figure 1. Larval mortality and pupicidal effect of *Dermatobia hominis* exposed to the methanolic extract of *Morinda citrifolia*. A. Larvicidal effect - prepupal period (Time 1 – T1). B. Pupicidal effect - Pupal period (Time 2 – T2). Kruskal-Wallis test *p<0.05.

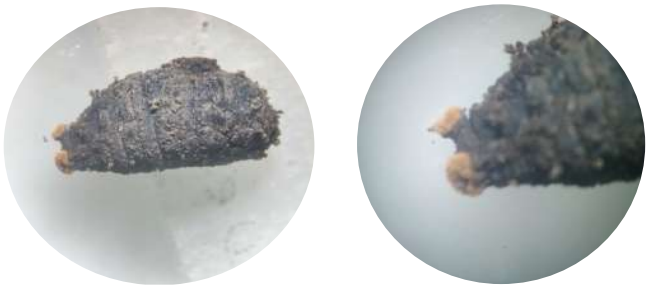
Results regarding the inhibition of the emergence of imagos, time 3 (T3), showed that all the L3 that survived the immersion test in the positive control group (ivermectin 1%), as well as in the negative group control (distilled water), pupated and finally emerged in the imago stage; while in groups G1, G2, and G3, the inhibition of imagos development from the pupal phase was 22% (95% CI 14.3 - 25.9), 24% (95% CI 22.4 - 27.5) and 25% (95% CI). 23.6 - 27.1), respectively; and in groups G4, G5, G6, and G7 the imagos emergence inhibition remained constant at 50% (95% CI 41.3 - 58.3), observing in these groups significant differences (p<0.05) compared to controls and groups G1 to G3. Likewise, only 10% (95% CI 6.9 - 12.3) and 20% (95% CI 13.2 - 27.4) of the imagos that emerged in groups G6 and G7, respectively, presented morphological deformations in the development of their head, being significant (p<0.05) the effect of abnormality in the development of the head of G7 group with respect to the lower concentrations of the extract (G1 to G5) and control groups, where no individuals with these alterations were observed (Figure 2B).



2.A.i Pupa lacking anterior spiracles (G7).



2.B.i Frontal and lateral views of imago abnormal head (G7).



2.A.ii Pupa with anterior spiracles (G1).

2.B.ii Frontal and lateral views of imago with normal head (G1).

Figure 2. Photographs of T2 (pupa phase) and T3 (imago phase) demonstrating the contrast in morphological changes between groups. **A.i** Experimental Group 7 (460 mg/mL) lacking anterior spiracles and **A.ii** Experimental Group 1 (10 mg/mL) with no morphological alterations. **B.i** Experimental imago Group 7 (460 mg/mL) with morphological deformations in the development of their head. **B.ii.** Experimental imago Group 1 (10 mg/mL) with no morphological head alterations.

The preliminary phytochemical screening of the methanolic extract of the *Morinda citrifolia* ripe fruit through the colorimetry technique allowed us to establish the presence of groups of secondary metabolites such as flavonoids, coumarins, cardiotonic glucosides, tannins, quinones, and steroids (Table 1).

Table 1. Characterization of the groups of secondary metabolites in the methanolic extract of *Morinda citrifolia.*

Groups of secondary metabolites	Colorimetric test/result
Alkaloids	Dragendorf´s reagent (-) Valser´s reagent (-) Mayer´s reagent (-)
Coumarins	Kedde´s reaction (+) UV (+)
Flavonoids	Shinoda´s reagent (+)
Tannins	Ferric Chloride 10% (+)
Steroids	Liebermann-Burchard test (+)
Quinones	Zinc powder (+) HCl (+)
Anthraquinones	Bornträger-Kraus test (-)
Cardiotonic Glucosides	Kedde´s reaction (+)

-: Absence, +: Presence

DISCUSSION

M. citrifolia is a plant widely studied for its medicinal properties (32, 33), considered one of the main sources of biologically active compounds (34). In traditional human medicine, it has been used to treat diabetes, arthritis, hypertension, among others (35), this has allowed to investigate and establish important beneficial properties on human and animal health. Actually, it has been reported that the influence of this plant has increased to the point of improving cattle (36) and broiler birds' growth (37). It is widely known that the use of plants for medicinal purposes is a practice developed for years (38).

Arthropods, mainly insects, mites, and ticks, represent the most economically important group of bovine ectoparasites due to the direct effect associated with heavy infestations affecting health and food production (39). For their treatment, ectoparasiticides are also used, these veterinary drugs have revealed the need to seek strategies that limit the side effects of their indiscriminate use in the context of food safety, global change, animal welfare, and environmental health (40). Indeed, *M. citrifolia* essential oil has been studied as a sustainable alternative for controlling phytopathogens and insect pests (41).

One of the main concerns regarding the use of plant extracts is the knowledge about their toxicity on animals and *M. citrifolia* is not an exception (42, 43). Artega *et al.* (44) carried out the acute toxicological classification of a product derived from the pulverized dried fruit of *M. citrifolia* in rats, concluding that (according to the class toxicity method) it was not classified, since the acute median lethal dose (LD_{50}) was greater than 2 g/kg, showing that 100% of animals survived. On the other hand, *M. citrifolia* has been referenced with potential as herbal medicine for treating livestock diseases in indigenous cultures (45). In the Meta department in Colombia, rural communities, in general, take advantage of the qualities of medicinal plants that have traditionally been applied to both humans and animals. According to the World Health Organization, at least 80% of the population of countries in development uses indigenous practices for the control and treatment of various diseases that affect livestock (46).

To our knowledge, there are no ethnopharmacological validation studies of *M. citrofolia* against *D. hominis.* Therefore, phytopharmacological investigations of *M. citrofolia* used to control other parasitic agents in animal production systems, are mentioned. Nápoles *et al.* (47), evaluated the *in-vitro* efficacy of *M. citrifolia* for the control of *Rhipicephalus microplus* (Acari: *Ixodidae*), emphasizing the medicinal properties attributed to its fruits, root, bark, seeds, and leaves previously described with astringent, analgesic and anthelmintic anti-inflammatory activities, concluding that the maximum efficacy of *M. citrifolia* was 20.6% in the control of this tick, using the concentration of 10%. The presence of ticks, especially *R. microplus,* causes skin damage, a predisposing factor for cutaneous myiasis, including the BCD (48), the results obtained by Nápoles *et al.* (47) allow us to infer that the use of *M. citrifolia* could be an alternative for the control of ectoparasites.

In Colombia, environmental conditions are conducive to the development and survival of both, internal and external parasites (49), in the Meta department, *D. hominis* is widely distributed, it causes obligatory myiasis on the animal since the parasite depends on the host to complete its life cycle (50) and generates pain stress caused by the movement of larvae within the nodules (17), the larvae actively penetrate the skin of the host and begin their development in the subcutaneous tissue (51).

Currently, there are no studies that directly evaluate the action of *M. citrifolia* on the main vectors of *D. hominis* eggs. In Colombia, vectors such as *Anopheles boliviensis, Haemagogus equimus, Mansonia* sp, *Psorophora feroz, Wycomya* sp. *Stomoxis calcitrans, Anopheles apicimacula and Cryptolucilia* sp, have been described (52). However, there is evidence of the effects of the extract of *M. citrifolia* used in the control of invertebrates, such as those reported by Sánchez (53) who demonstrated the toxic action of noni extracts on larval stages of *Spodoptera frugiperda*. On the other hand, Kovendan *et al.* (54) reported mosquitocidal activity in the development phases of the malaria vector, *Anopheles stephensi,* the dengue vector, *Aedes aegypti,* and the filarial vector, *Culex quinquefasciatus.* According to these results, there is the possibility of generating the same effects not only on *D. hominis* but also on its main phoretic vectors, since many of these studies have obtained results on invertebrates of the Diptera order, like those mentioned as phoretic vectors of *D. hominis* eggs, playing a fundamental role in the development of the disease. These results were evident in Diptera of the *Drosophilidae* family, as well as mosquito vectors of tropical diseases, ants, and bees where the authors have mentioned the octanoic acid (C8) as the main toxic compound of this plant on the studied insects; and the hexanoic acid (C6) with great advantages as an insecticide and mosquito repellent (55).

In our study, developmental anomalies were observed in the head of the imagos (Figure 2B) that emerged from the larvae exposed to 40% and 46% concentrations of the methanolic extract of *M. citrifolia,* hence the hypothesis to be verified in subsequent studies, to test if the effect that is reported by C8 and C6 complexes on invertebrates (55 56) is also verified in the different *D. hominis* life stages.

On the other hand, in our study, the maximum larvicidal efficacy on the L3 phase of *D. hominis was* 60% (95% CI 56.8 - 67.3) achieved with the highest concentration (460 mg/mL) of the methanolic extract of the *M. citrofolia* ripe fruit (Figure 1A) and the maximum pupicidal efficacy was 70% (CI 95% 67.1 – 76.7) achieved as well with the highest concentration (460 mg/mL) (Figure 1B). Since there are no other studies about this larvicidal and pupicidal effect on *D. hominis,* it is necessary to carry out more studies (through bioguided fractionation) in order to know the efficacy of the crude extract and its fractions. Kovendan *et al.* (57) have reported positive results with larvicidal and pupicidal activity of the ethanolic extract of *M. citrifolia* leaves from India, against the malaria vector *Anopheles stephensi,* in a range of 18.3 to 97.8 mg/L and Opoku-Bamfoh et al (58), found that emulsified *Morinda citrifolia* seed oil had LC_{50} = 68.3 (95% CI 53.3 - 79.2) and LC_{90}=130.9 (95% CI 37.9 - 42.3), against *Anopheles gambiae.* Additionally, according to Rahayu *et al.* (59), the leaf extract of this plant at 20% can be used as bioinsecticide to control populations of german cockroaches, which have been resistant to commercial insecticides.

In addition, the essential oil of the *M. citrifolia* fruit was used to evaluate the larvicidal effect on the *Aedes aegypti* mosquito (Diptera: *Culicidae*), demonstrating the insecticidal action of this oil with a lethal concentration (LC) 50 (LC_{50}) of 151.9 mg/L and LC_{90} of 195.5 mg/L (60) and Almeida *et al.* (42) found that *M. citrifolia* fruit juice exhibited antileishmanial activity against *Leishmania amazonensis* amastigotes. These results broaden the vision of the effect of *M. citrifolia,* since despite the fact that various reports include wide differences in the effective concentrations, the expected effect as a therapeutic alternative for the control of parasitosis, continues to be positive.

According to the data reported by the Instituto Colombiano Agropecuario (ICA) (61), by 2021, the Meta department has a livestock inventory that exceeds two million animals, including it as one of the main areas of concentration of bovine animals in Colombia. This has encouraged the need to implement economic strategies that complement the comprehensive health management of farm animals. From this perspective, this study places *M. citrifolia* as a promising plant adapted to the region, which could be a therapeutic alternative in the parasite control of *D. hominis* in cattle.

Studies have given evidence regarding the biologically active components *of M. citrifolia* (62 - 65), this has also been demonstrated in our results, through the phytochemical screening of the methanolic extract of *M. citrifolia* ripe fruit, collected between the foothills and highlands of the Meta department. Through the colorimetric technique, we established the presence of secondary metabolites such as flavonoids, coumarins, cardiotonic glucosides, tannins, quinones, and steroids (Table 1). Most of these secondary metabolites have been previously studied in different settings and have been widely reported and validated, especially with immune-enhancing effects including antibacterial, anti-inflammatory, anticancer, and antioxidant activity (63, 64).

CONCLUSION

This study provides a first *in-vitro* approach related to the effects of the methanolic extract of the *M. citrifolia* ripe fruit against the life cycle of *D. hominis;* which could be a promising strategy to be implemented as a therapeutic alternative within the integrated control of bovine cutaneous dermatobiosis in animal production systems in the tropics.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: RFQ, DAC, and DAJ; methodology: RFQ, DAC, AEG, and DAJ; writing (original draft preparation): DAJ and LNP; review and editing: DAJ, LNP, and LCL. All authors have read and agreed to the published version of the manuscript.

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