

***In vitro* biological activity of hydroalcoholic extracts from *Caesalpinia ferrea* and *Lippia organoides* on adults of *Bovicola caprae* (Gurlt, 1843) (Mallophaga: Trichodectidae)**

[Atividade biológica in vitro de extratos hidroalcoólicos de *Caesalpinia ferrea* e de *Lippia organoides* sobre adultos de *Bovicola caprae* (Gurlt, 1843) (Mallophaga: Trichodectidae)]

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ABSTRACT

The conventional control of *Bovicola caprae* consists of using chemical insecticides, which often becomes ineffective due to the development of resistance, stimulating the search for alternative control methods. This study evaluated the *in vitro* bioinsecticidal potential of hydroalcoholic extracts from *Caesalpinia ferrea* and *Lippia organoides* against adult *B. caprae*. The extracts of *C. ferrea* and *L. organoides* were obtained from the fruits and leaves, respectively. Specimens of *B. caprae* (80 per group) were exposed to five different concentrations of each extract. The tests were conducted in duplicate, using distilled water (negative control) and deltamethrin (positive control) as control groups. Mortality was assessed at 2h, 4h, 6h, 12h, 24h, and 48h after exposure to the products. Both extracts caused mortality in the lice. Total mortality was observed after 6h of exposure to *L. organoides* extract at 130 mg/mL. *C. ferrea* presented an LC₅₀ of 67.82mg/mL up to 4h and caused total mortality at a concentration of 78.75 mg/mL from 12h after exposure to the hydroalcoholic extracts. Due to the insecticidal activity observed, both extracts are considered promising for the control of this ectoparasite.

Keywords: phytotherapy, ectoparasites, pediculosis, goats, bioactives

RESUMO

O controle convencional de *Bovicola caprae* consiste na utilização de inseticidas químicos, que muitas vezes se tornam ineficientes frente ao desenvolvimento de resistência, estimulando a busca por métodos alternativos de controle. Neste estudo, avaliou-se o potencial bioinseticida in vitro de extratos hidroalcoólicos de *Caesalpinia ferrea* e de *Lippia organoides* sobre adultos de *B. caprae*. Os extratos de *C. ferrea* e *L. organoides* foram produzidos a partir dos frutos e das folhas, respectivamente. Espécimes de *B. caprae* (80 por grupo) foram expostos a cinco diferentes concentrações de cada extrato. Os testes foram realizados em duplicata, e água destilada (controle negativo) e deltametrina (controle positivo), foram utilizadas como grupos controle. A mortalidade foi avaliada a 2h, 4h, 6h, 12h, 24h e 48h após a exposição aos produtos. Ambos os extratos causaram mortalidade nos piolhos. A mortalidade total foi observada em 6h de exposição ao extrato de *L. organoides* em 130mg/mL. *C. ferrea* apresentou LC₅₀ de 67.82mg/mL até 4h e causou mortalidade total na concentração 78,75mg/mL a partir de 12h após a exposição aos extratos hidroalcoólicos. Pela atividade inseticida evidenciada, ambos os extratos são considerados promissores para o controle deste ectoparasito.

Palavras-chave: fitoterapia, ectoparasitos, pediculose, caprinos, bioativos

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INTRODUCTION

Caprine pediculosis leads to economic losses and affects herd health worldwide, causing dermatological issues and a decrease in animal production due to stress-related factors and consequent growth reduction (Iqbal *et al.*, 2014; Prelezov; Nizamov, 2020). In Brazil, chewing lice are considered the most frequent ectoparasites in goat farming. Particularly in the Northeast region, *Bovicola caprae* (syn. *Damalinia caprae*) has been a challenge for livestock farmers, with the proliferation of these lice observed during dry periods or in herds where animals are subjected to inadequate feeding and management conditions (Santos *et al.*, 2006).

The conventional control of *B. caprae* consists of using synthetic neurotoxic insecticides, particularly organophosphates and pyrethroids. However, in recent years, the progressive development of resistance due to mutations in sodium channels has contributed to the inefficacy of these products (Gazafi *et al.*, 2023). Additionally, the use of insecticides poses a challenge to One Health, given their environmental impact and residual effects on the environment, as well as animal and human health (Lu *et al.*, 2019; Hołyńska-Iwan; Szewczyk-Golec, 2020). Thus, there is an increasing search for new control methods, particularly botanical alternatives, such as plant extracts and essential oils containing bioactive compounds with insecticidal properties (Pohlit *et al.*, 2011; Štrbac *et al.*, 2023).

In this context, *Caesalpinia ferrea* Mart. (Fabaceae), commonly known as "pau-ferro", is a plant native to South America and widely distributed in the northern and northeastern regions of Brazil. It has extensive pharmacological properties, particularly insecticidal activity (Andrade *et al.*, 2020; Macêdo *et al.*, 2020). The larvicidal effect of *C. ferrea* extracts on *Aedes aegypti* (Andrade *et al.*, 2020) and its acaricidal activity against *Rhipicephalus (Boophilus) microplus* (Rosado-Aguilar *et al.*, 2017) demonstrate the potential of bioactive compounds derived from *C. ferrea* metabolism, such as flavonoids, terpenoids, tannins, steroids, saponins, polyphenols, and other phenolic compounds (Macêdo *et al.*, 2020; Silva *et al.*, 2023).

Lippia origanoides Cham. (Verbenaceae) is also widely used due to its rich composition of bioactive compounds, such as carvacrol and thymol (Oliveira *et al.*, 2007). Its extracts and essential oils have shown activity against arthropods, e.g., *Ae. aegypti* (Andrade *et al.*, 2020) and *R. (B.) microplus* (Chagas *et al.*, 2016), as well as significant potential against gastrointestinal nematodes in goats (Souza *et al.*, 2020) and antimicrobial properties, creating bacterial inhibition zones (Oliveira *et al.*, 2007).

Although studies report the insecticidal potential of plants such as *C. ferrea* and *L. origanoides*, evaluations on lice are scarce. Given the impact of ectoparasites on goat production in northeastern Brazil and the parasitic resistance exhibited by these arthropods, the search for alternative control methods is essential. Thus, this study aimed to evaluate the *in vitro* bioinsecticidal potential of *C. ferrea* and *L. origanoides* extracts against *B. caprae*.

ETHICAL ASPECTS

All procedures involving animals were conducted in accordance with ethical standards and were approved by the Animal Use Ethics Committee of the Federal Rural University of Pernambuco (CEUA-UFRPE), under license no. 8717300420.

MATERIALS AND METHODS

Botanical material and preparation of hydroethanolic extracts

The plants *Caesalpinia ferrea* and *Lippia origanoides* were collected in the city of Recife, Pernambuco, Brazil. These specimens were used to prepare herbarium vouchers, identified, and registered in official collections. *C. ferrea* was incorporated into the Geraldo Mariz Herbarium at the Federal University of Pernambuco (registration no. 69656), and *L. origanoides* was deposited in the Dárdano de Andrade Lima Herbarium (registration no. 92547) at the Agronomic Institute of Pernambuco.

To produce hydroalcoholic extracts, the fruits of *C. ferrea* and the leaves of *L. origanoides* were dehydrated in a circulating-air oven at 40°C and then ground in a knife mill. The resulting plant material was macerated in 1L of 70% ethanol for

72 hours to obtain the extracts. The solutions were then filtered and concentrated using a rotary evaporator under reduced pressure at 45°C and 120 rotations per minute (RPM) and stored under refrigeration (Andrade *et al.*, 2020).

From the crude extracts, solutions at different concentrations were prepared by dilution in distilled water. The concentrations were used: *C. ferrea* (13.13, 26.25, 52.50, 78.75, and 105.0mg/mL) and *L. origanoides* (16.25, 32.50, 65.0, 97.5, and 130.0 mg/mL). The selection of these concentration ranges was based on previous studies that demonstrated insecticidal and/or larvicidal activity of similar plant species (Andrade *et al.*, 2020; Chagas *et al.*, 2016).

The use of fractional values aimed to allow a comparative assessment of the dose-response relationship. It is acknowledged, however, that employing different concentration ranges for each extract may limit the validity of direct comparisons between treatments. Therefore, it is emphasized that comparisons between the extracts were conducted in an exploratory manner, and the study did not intend to establish definitive conclusions regarding the superiority of one over the other.

The solutions were prepared using a properly calibrated analytical balance, and the final concentrations were determined based on the exact mass of crude extract diluted in distilled water, ensuring accuracy and reproducibility of the experiments.

Collection and identification of Bovicola caprae

The biological activity of the extracts was evaluated on adult specimens of *Bovicola caprae* collected, using anatomical forceps, from goats that were naturally infested and had not been treated with synthetic insecticides for at least 30 days. All lice were collected from goats on a private property located in the metropolitan region of Recife, Pernambuco, Brazil. The property was selected for convenience (Reis, 2003). The specimens were stored in plastic containers and transported to the Laboratory of Parasitic Diseases at the Federal Rural University of Pernambuco for experimental setup. These insects were identified using the keys of Tuff (1977) and Furman and Catts (1977).

In vitro bioassay on adult Bovicola caprae

For the immersion tests, 560 adult specimens (approximately 30 minutes post-collection) were used, divided into treatment and control groups, in such a way that each treatment received the same number of lice. A total of 80 lice were allocated to each treatment group with hydroethanolic extract, 80 to the negative control group with distilled water, and 80 to the positive control group with deltamethrin 25g (Butox®), following the manufacturer's recommended dilution of 1 mL per liter of water. The experiment was conducted in duplicate, with 40 specimens per plate. Lice were immersed for 5 minutes in 30 mL of the respective treatment solutions, as well as in the control solutions. After immersion, the specimens were dried on filter paper, placed in plastic containers, maintained at a temperature of $26 \pm 2^\circ\text{C}$, and monitored for mortality at 2h, 4h, 6h, 12h, 24h, and 48h, using a stereomicroscope to assess the motility of each individual specimen during a two-minute observation period per plate.

DATA ANALYSIS

Descriptive statistics were initially performed to obtain absolute and relative frequencies. The Friedman test was used to evaluate louse mortality at different time points after exposure to the extracts. The Kruskal-Wallis test was applied to compare the different concentrations. Multiple comparisons for both extracts were conducted using the Mann-Whitney test. The median lethal concentration (LC_{50}) and the respective 95% confidence intervals (CI 95%) were determined using the Probit model.

A 5% margin of error was considered for statistical tests. Data analysis was performed using BioEstat version 5.3 and MedCalc version 14.8.1.

RESULTS AND DISCUSSION

The insecticidal activity of the hydroethanolic extracts of *C. ferrea* (Tab. 1) and *L. origanoides* (Tab. 2) on adult *B. caprae* was observed with an increase in concentrations and evaluation time ($p < 0.05$). The mortality rate of lice observed in the control group using distilled water was consistently below 10% during the evaluations, which is considered a natural occurrence in insecticidal efficacy tests, with no need to adjust

the values for the groups exposed to the botanical extracts (Abbot, 1925).

The extract of *C. ferrea* at the highest concentration (105.0 mg/mL) caused total mortality of the lice after 12 hours of exposure (Table 1). In contrast, the extract of *L. origanoides* caused total mortality of the lice within 6 hours at its highest concentration (130.0 mg/mL). However, due to the use of different concentration ranges for each extract, this observation does not allow for a direct comparison of the speed of action between the two botanical species. Therefore, such differences should be interpreted cautiously and do not imply superiority of one extract over the other. The positive control (deltamethrin)

achieved total mortality of the lice after 4 hours of the experiment.

At the lowest concentrations tested, both extracts showed lower mortality rates. However, partial insecticidal activity was still observed, indicating a dose-dependent effect. According to Drummond *et al.* (1973), products are considered effective when they cause mortality rates above 95%. Therefore, although this threshold was not reached at the lowest concentrations, the observed mortality still suggests a biological effect of the extracts.

However, the average mortality rates obtained at different concentrations of *C. ferrea* differed from each other since the first two evaluations (2h and 4h) (Table 1).

Table 1. Average percentage and standard deviation of *Bovicola caprae* mortality over 48 hours after exposure to the hydroethanolic extract of *Caesalpinia ferrea*

Concentration (mg/mL)	Time (h)						p – value
	2	4	6	12	24	48	
Negativecontrol	0.0± 0.0	0.0± 0.0	1.3 ± 1.78	2.5 ± 1.78	2.5 ± 1.8	2.5 ± 1.8	p ⁽¹⁾ = 0.101
Positive control	77.5± 3.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.00 ± 0.0	100.0 ± 0.0	p ⁽¹⁾ = 0.075
13.13	0.00± 0.0 ^{aA}	10.0 ± 3.5 ^{aAB}	16.25 ± 1.8 ^{aB}	31.25 ± 8.8 ^{aC}	40.0 ± 3.5 ^{aCD}	47.50 ± 3.5 ^{aD}	p ⁽¹⁾ = 0.075
26.25	10.00± 3.5 ^{bA}	20.00 ± 7.1 ^{bB}	31.25 ± 8.8 ^{bC}	38.75 ± 5.3 ^{aD}	52.50 ± 3.5 ^{bE}	62.50 ± 0.0 ^{bF}	p ⁽¹⁾ = 0.015
52.5	22.50±3.5 ^{cA}	33.75 ± 5.3 ^{cB}	52.50 ± 3.5 ^{cC}	61.25 ± 1.8 ^{bD}	77.50 ± 3.5 ^{cE}	88.75 ± 1.8 ^{cF}	p ⁽¹⁾ = 0.015
78.75	40.00± 3.5 ^{dA}	68.75 ± 1.8 ^{dB}	92.50 ± 7.1 ^{dC}	100.0 ± 0.0 ^{cD}	100.0 ± 0.0 ^{dD}	100.0 ± 0.0 ^{dD}	p ⁽¹⁾ = 0.075
105	51.25± 5.3 ^{dE}	73.75 ± 1.8 ^{dB}	96.25 ± 5.3 ^{dC}	100.0 ± 0.0 ^{cC}	100.0 ± 0.0 ^{dC}	100.0 ± 0.0 ^{dC}	p ⁽¹⁾ = 0.003
p-value	p ⁽²⁾ =0.055	p ⁽²⁾ =0.055	p ⁽²⁾ = 0.061	p ⁽²⁾ =0.059	p ⁽²⁾ =0.054	p ⁽²⁾ =0.054	

⁽¹⁾ Friedman Test; ⁽²⁾ Kruskal Wallis Test; p < 0.05 indicates a significant association in the tests.

Distinct lowercase letters indicate significant differences in the multiple comparison tests among the different concentrations at a given time, whereas distinct uppercase letters indicate significant differences in the multiple comparison tests among the different times for a given concentration.

Total mortality of *B. caprae* exposed to *L. origanoides* occurred within 6 hours at the

highest concentration (130.0 mg/mL). However, total mortality was not observed in all treatment groups, the extracts exhibited partial insecticidal activity. Mortality rates below 95% still represent a biological effect. However, the hydroethanolic extract of *L. origanoides* showed efficacy, according to Drummond *et al.* (1973), only after 24 hours of exposure at concentrations of 65.0 and 97.5mg/mL (Table 2).

In vitro biological activity...

Table 2. Average percentage and standard deviation of *Bovicola caprae* mortality over 48 hours after exposure to the hydroethanolic extract of *Lippia origanoides*

Concentration (mg/mL)	Time (h)						p – value
	2	4	6	12	24	48	
Negative control	0.00± 0.0	0.00± 0.0	0.00± 0.0	0.00± 0.0	3.75 ± 1.8	5.00±3.5	p ⁽¹⁾ =0.078
Positive control	77.50 ± 3.5	100.00 ± 0.0	100.00± 0.0	100.00± 0.0	100.00 ± 0.0	100.00± 0.0	p ⁽¹⁾ =0.075
16.25	0.00± 0.0 ^{aA}	13.75 ± 1.8 ^{aB}	23.75± 1.8 ^{aC}	28.75± 1.8 ^{aD}	37.50 ± 0.0 ^{aE}	52.50± 3.5 ^{Af}	p ⁽¹⁾ =0.075
32.5	17.50 ± 7.1 ^{bA}	33.75 ± 5.3 ^{bB}	47.50± 0.0 ^{bC}	60.00± 3.5 ^{bD}	66.25± 1.8 ^{bDE}	73.75± 1.8 ^{bE}	p ⁽¹⁾ =0.075
65.0	27.50± 3.5 ^{bcA}	35.00 ± 3.5 ^{bA}	53.75± 5.3 ^{bB}	83.75± 5.3 ^{cC}	98.75 ± 1.8 ^{cD}	100.00± 0.0 ^{cD}	p ⁽¹⁾ =0.001
97.5	32.50 ± 3.5 ^{cA}	52.50 ± 3.5 ^{cB}	86.25± 15.9 ^{cC}	93.75± 8.8 ^{cdC}	100.00± 0.0 ^{cC}	100.00± 0.0 ^{cC}	p ⁽¹⁾ =0.001
130	55.00 ± 7.1 ^{dA}	91.25 ± 1.8 ^{dB}	100.00± 0.0 ^{cC}	100.00± 0.0 ^{dC}	100.00± 0.0 ^{cC}	100.00± 0.0 ^{cC}	p ⁽¹⁾ =0.075
p-value	p ⁽²⁾ =0.057	p ⁽²⁾ =0.061	p ⁽²⁾ =0.054	p ⁽²⁾ =0.060	p ⁽²⁾ =0.061	p ⁽²⁾ =0.054	

⁽¹⁾ Friedman Test; ⁽²⁾ Kruskal Wallis Test; p < 0.05 indicates a significant association in the tests.

Distinct lowercase letters indicate significant differences in the multiple comparison tests among the different concentrations at a given time, whereas distinct uppercase letters indicate significant differences in the multiple comparison tests among the different times for a given concentration.

The analysis of the LC₅₀ at 4 hours of exposure indicated values of 67.82mg/mL for *C. ferrea*

and 74.91 mg/mL for *L. origanoides* (Table 3). Although this result suggests a slightly higher insecticidal potential of the *C. ferrea* extract, the wide variation in the confidence interval observed for *L. origanoides* and the overlap between the intervals (61.47 - 74.91mg/mL for *C. ferrea* and 39.48 - 122.63mg/mL for *L. origanoides*) prevent this assertion between the two extracts at this exposure time.

Table 3. Estimates of the median lethal concentration (LC₅₀) and 95% confidence interval of the hydroethanolic extracts of *Caesalpinia ferrea* and *Lippia origanoides* on *Bovicola caprae*.

Hydroethanolic extract	Evaluation time (h)					
	2	4	6	12	24	48
CL ₅₀ (mg/mL) Confidence Interval 95%						
<i>Caesalpinia ferrea</i>	96.77 88.06-108.80	67.82 61.47-74.91	44.08 39.30-48.75	33.23 5.41-52.30	23.57 17.85-28.34	16.48 9.83-21.46
<i>Lippia origanoides</i>	120.45 86.93-327.30	74.91 39.48-122.63	46.67 16.10-68.45	30.42 23.16-36.51	23.20 18.71-26.97	15.50 8.17-20.16

Lice (Malophaga), despite being permanent parasites, can survive outside the host for more than 48 hours (Heath, 1973; Farias *et al.*, 2017),

which indicates that the average mortality rates of *Bovicola caprae* at different concentrations and evaluation times in the present study

demonstrate the insecticidal activity of *C. ferrea* and *L. origanoides* extracts.

Although plant extracts and essential oils from various plants have insecticidal activity against arthropods of veterinary importance, there is a lack of research focused on the use of phytotherapeutics for controlling lice populations, especially *B. caprae*, making this is among the first reports of the biological activity of *C. ferrea* and *L. origanoides* against this species. However, among the few studies on controlling this ectoparasite with botanical options, the essential oil from the seed of *Carapa guianensis*, an Amazonian tree, showed promise against *B. caprae* at low concentrations and in a short time (Farias et al., 2017). In the present study, only the highest concentrations of both extracts reached mortality rates $\geq 95\%$ (Drummond et al., 1973), thus being considered effective as insecticides. At lower concentrations, partial effects were observed, with mortality rates below this threshold. This difference may be attributed to variations in chemical composition, the type of extract tested, and the experimental conditions adopted. These findings, however, reinforce the insecticidal potential of native plants from the Brazilian flora.

In addition, *in vitro* studies evaluated the effect of the crude extract of *Allium sativum* on *B. caprae*, showing 100% mortality at concentrations of 100 mg/mL and 50 mg/mL in 32 and 48 hours, respectively (Lakshmanan et al., 2013). This highlights the importance of conducting additional evaluations over extended periods and at different concentrations of the same plant, to better understand the persistence of the insecticidal effect. Furthermore, *in vivo* tests are needed to assess both the toxicity to the host and the lethality of the phytotherapeutic against the parasite (Lakshmanan et al., 2013).

Moreover, the discrepancy between the insecticidal effect of the *C. ferrea* and *L. origanoides* extracts is due to, among other factors, the origin of the plant, the bioactive compounds present in each extract and their action on *B. caprae*, differences in the tested concentrations, as well as the unique insecticidal potential of different parts of the same plant, the nature of the products evaluated, and the chemical composition of the solvent used (Ambrozim et al., 2006; Nalamwar et al., 2009).

Hydroalcoholic extracts of *C. ferrea* fruits are known for the presence of phenolic compounds and catechins with insecticidal action, effective against *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) (Lopes et al., 2018), while the fruit peel has repellent action against Calliphoridae flies (Fernandes et al., 2016). The oil from aerial parts of *L. origanoides*, rich in phellandrene, shows repellent activity against adults of *Ulomoides dermestoides* (Coleoptera: Tenebrionidae) (Caballero-Gallardo et al., 2022), and the hydroalcoholic extract from its leaves has larvicidal effects on *Aedes aegypti*, attributed to the major presence of thymol (Andrade et al., 2020).

The bioinsecticidal action of different concentrations of hydroalcoholic extracts from *C. ferrea* and *L. origanoides* emerges as an alternative for lice control, as populations of *B. caprae* with pyrethroid resistance alleles have already been reported in several regions of the world (Gazafi et al., 2023). Ivermectin, a macrolide lactone widely used *in vivo* via parenteral administration for ectoparasite control in ruminants, has demonstrated pediculicidal effects against *B. caprae*. However, despite its broad use, the average protection period provided by this drug is less than 30 days, which limits its effectiveness in long-term control of new infestations. This reinforces the need for new, more sustainable prophylactic alternatives, such as plant-based bioinsecticides (Dimri et al., 2019).

CONCLUSION

The hydroethanolic extracts of *Caesalpinia ferrea* and *Lippia origanoides* demonstrated *in vitro* bioinsecticidal activity against *Bovicola caprae*, with progressive increases in mortality according to exposure time and concentration. Only the highest concentrations of both extracts achieved mortality rates $\geq 95\%$, which are considered effective as insecticides, while lower concentrations produced only partial effects. The results suggest that both extracts are promising for the control of this ectoparasite, but emphasize the need for additional studies, particularly *in vivo* assays and tests with standardized concentrations, to confirm the potential and applicability of these compounds in the control of caprine pediculosis.

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CONTRIBUTORS

TAS Andrade: study conception, experimental procedures, material collection, data analysis and manuscript writing; EHA Silva: data analysis, manuscript writing, manuscript review; TRM Silva, LO Macedo, RAN Ramos: manuscript writing, manuscript review; ETN Farias, GC Jimenez: material collection, experimental procedures. LC Alves, MAG Faustino: experimental procedures, manuscript review; GA Carvalho: study conception, data analysis, manuscript review and approval of the final version to be published.

DATA AVAILABILITY STATEMENT

The research data are available within the article itself.

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