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LICHEN EXTRACTS FROM PARMOTREMA SPECIES DO NOT EXHIBIT ACARICIDAL EFFECTS ON RESISTANT RHIPICEPHALUS MICROPLUS LARVAE

EXTRATOS DE LIQUENS DE ESPÉCIES DE PARMOTREMA NÃO EXIBEM EFEITOS ACARICIDAS EM LARVAS RESISTENTES DE RHIPICEPHALUS MICROPLUS

EXTRACTOS DE LÍQUENES DE ESPECIES DE PARMOTREMA NO PRESENTAN EFECTOS ACARICIDAS EN LARVAS RESISTENTES DE RHIPICEPHALUS MICROPLUS

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ABSTRACT

Lichens, symbiotic associations between a mycobiont and a photosynthetic partner, produce a wide range of secondary metabolites with documented antifungal, antibacterial, anti-inflammatory, anticoagulant, and antiparasitic properties. Within this group, the family Parmeliaceae is one of the most diverse, and several of its species are known to synthesize biologically active lichen acids. To evaluate the potential acaricidal activity of these compounds, methanolic and hexanic extracts were prepared from the thallus most abundant Parmotrema (P. latissimum, P. mesotropum, and P. rubifaciens) species collected in the Cerrado areas of Itaguatins/ Tocantins, Brazil. Biological activity was assessed through larval immersion assays following Klafke (2006), using resistant larvae of Rhipicephalus microplus (Jaguar strain) exposed to extract concentrations ranging from 0.4 to 2.0 mg/ML, serial dilutions. Under the conditions tested, all extracts exhibited 0% larval mortality, indicating an absence of acaricidal activity against the resistant tick lineage at the evaluated concentrations.

Keywords: Bioprospection. Cattle Tick. Cerrado. Liquenized Fungi. Parmeliaceae. Secondary Metabolites.

RESUMO

Os liquens, associações simbióticas entre um micobionte e um parceiro fotossintético, produzem uma ampla variedade de metabólitos secundários com propriedades antifúngicas, antibacterianas, anti-inflamatórias, anticoagulantes e antiparasitárias já documentadas.

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Dentro desse grupo, a família Parmeliaceae é uma das mais diversas, e várias de suas espécies são conhecidas por sintetizar ácidos liquênicos biologicamente ativos. Para avaliar o potencial acaricida desses compostos, extratos metanólicos e hexânicos foram preparados a partir do talo das espécies de Parmotrema mais abundantes (P. latissimum, P. mesotropum e P. rubifaciens) coletadas em áreas de Cerrado em Itaguatins, Tocantins, Brasil. A atividade biológica foi avaliada por meio de ensaios de imersão larval conforme Klafke (2006), utilizando larvas resistentes de Rhipicephalus microplus (linhagem Jaguar) expostas a concentrações de extrato variando de 0,4 a 2,0 mg/mL, em diluições seriadas. Nas condições testadas, todos os extratos apresentaram 0% de mortalidade larval, indicando ausência de atividade acaricida contra a linhagem resistente do carrapato nas concentrações avaliadas.

Palavras-chave: Bioprospecção. Carrapato do Gado. Cerrado. Fungos Liquenizados. Parmeliaceae. Metabólitos Secundários.

RESUMEN
Los líquenes, asociaciones simbióticas entre un micobionte y un socio fotosintético, producen una amplia variedad de metabolitos secundarios con propiedades antifúngicas, antibacterianas, antiinflamatorias, anticoagulantes y antiparasitarias ya documentadas. Dentro de este grupo, la familia Parmeliaceae es una de las más diversas, y varias de sus especies son conocidas por sintetizar ácidos liquénicos biológicamente activos. Para evaluar el potencial acaricida de estos compuestos, se prepararon extractos metanólicos y hexánicos a partir del talo de las especies de Parmotrema más abundantes (P. latissimum, P. mesotropum y P. rubifaciens) recolectadas en áreas de Cerrado en Itaguatins, Tocantins, Brasil. La actividad biológica se evaluó mediante ensayos de inmersión larvaria según Klafke (2006), utilizando larvas resistentes de Rhipicephalus microplus (linaje Jaguar) expuestas a concentraciones de extracto que variaban de 0,4 a 2,0 mg/mL, en diluciones seriadas. En las condiciones evaluadas, todos los extractos presentaron un 0% de mortalidad larvaria, indicando ausencia de actividad acaricida contra el linaje resistente de la garrapata a las concentraciones probadas.

Palabras clave: Bioprospección. Garrapata del Ganado. Cerrado. Hongos Liquenizados. Parmeliaceae. Metabolitos Secundarios.

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1 INTRODUCTION

Lichens, symbiotic organisms formed by the association between a mycobiont and a photosynthetic partner, produce a wide array of secondary metabolites collectively known as lichen acids (Elix, 1996). These compounds play key ecological and physiological roles, including antifungal, antibacterial, anti-inflammatory, anticoagulant, and antiparasitic activities, making them highly relevant from a medicinal and biotechnological perspective (Dias et al., 2023).

The production of these metabolites also contributes to the lichens' defense against herbivory and enhances their ability to colonize a wide range of substrates (Morales et al., 2009). In addition to their ecological significance, lichen-derived substances hold considerable economic value and are used in the cosmetic and perfume industries (Xavier-Filho et al., 2006). More than 800 secondary metabolites have been identified in lichen-forming fungi, and the concentration and distribution of these compounds vary according to thallus structure and species (Elix, 2014).

The cattle tick *Rhipicephalus microplus* is one of the most significant parasitic threats to bovine production systems worldwide. Beyond transmitting pathogenic agents, infestations result in substantial economic losses due to reduced weight gain, decreased milk and calf production, and damage to hides (Alonso, 1992; Grisi et al., 2002).

Chemical control, introduced in Australia in the late nineteenth century (Wharton, 1980), quickly became the primary strategy for managing this parasite and remains the dominant method in many regions (Oliveira, 1999). However, improper acaricide use, including unplanned treatment schemes, inadequate monitoring of chemical concentrations in plunge dips, homemade pour-on mixtures, and indiscriminate application of the same active ingredients against other ectoparasites such as the horn fly, has accelerated the selection of resistant *R. microplus* populations (Charles & Furlong, 1996).

This growing resistance crisis underscores the urgent need for new acaricides with novel modes of action. In this context, natural products have emerged as a promising alternative, offering structurally diverse bioactive molecules with the potential to interact with biological targets not yet exploited by conventional acaricides (Prance, 1991; DeWitt, 1994). Lichen secondary metabolites, given their chemical diversity and documented biological properties, represent an underexplored but potentially valuable resource in the search for new tick-control agents.

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Considering the bioactive potential of secondary metabolites produced by lichens and the urgent need for sustainable alternatives for developing new acaricides, this study aimed to evaluate the acaricidal activity of lichen extracts from three *Parmotrema* species on the lethality of *Rhipicephalus microplus* larvae.

2 METHODOLOGY

Lichen thallus were collected in an area of typical *Cerrado sensu stricto* vegetation at Fazenda São Paulo, in the municipality of Itaguatins (5°44'58"S, 47°32'21"W), Tocantins, northern Brazil, within the Bico do Papagaio mesoregion. Three species of lichenized fungi from the family Parmeliaceae were selected for extract preparation: *Parmotrema latissimum* (Fée) Hale, *Parmotrema mesotropum* (Müll. Arg.) Hale, and *Parmotrema rubifaciens* (Hale) Hale. These species were chosen based on the availability of specimens and the greater amount of fresh biomass obtained.

For the chemical characterization of the species, only qualitative analyses were performed. Spot tests were applied to both the cortex and medulla of the thallus using standard color reagents, including K (10% potassium hydroxide), C (40% sodium hypochlorite), KC (K followed by C), and P (para-phenylenediamine). In addition, thin-layer chromatography (*TLC*) was conducted following established lichenological protocols, employing solvent systems A (toluene, dioxane, and acetic acid in a ratio of 170:45:5) and C (toluene and acetic acid) for metabolite separation. Complementary microcrystallization tests were performed to support the identification of characteristic secondary metabolites according to conventional methodologies (Huneck & Yoshimura, 1996; Bungartz, 2002; Orange et al., 2010; Elix, 2014).

Each specimen was cleaned, ground using an electric grinder, and weighed separately on an analytical balance. For extract preparation, 10 g of fresh material from each species were transferred to Falcon tubes and subjected to sequential extraction with methanol followed by hexane, in a 1:5 (w/v) ratio. The maceration process was carried out for 30 minutes in a sonication water bath. After maceration, the extracts were filtered through filter paper, and the liquid fractions were concentrated in a rotary evaporator until complete solvent removal. The resulting dry extracts were weighed and stored in glass containers for subsequent bioassays (Figure 1).

Serial dilutions of the dry lichen extracts were prepared in a solution composed of 30% ethanol and 2% Triton X-100. Ten concentrations were obtained for each extract, ranging

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from 2.0 mg/mL (highest concentration) to 0.4 mg/mL (lowest concentration). The ethanol–Triton solution (30%/ 2%) was used as the negative control.

Figure 1

Lichen extracts (methanolic and hexane) obtained

Larval immersion tests were performed following the methodology described by Klafke et al. (2006). For each concentration, 1 mL of extract solution was transferred to microtubes, and approximately 500 live larvae of the highly resistant Jaguar strain of *Rhipicephalus microplus* were added. The tubes were closed, gently agitated, and kept under this condition for 10 minutes. The larvae were then removed, placed on filter paper to dry, and subsequently transferred (~100 larvae per sample) to 8.5 × 7.5 cm filter-paper envelopes sealed with plastic clips.

All envelopes were incubated at 27 ± 1 °C and approximately 80% relative humidity for 24 hours. After incubation, live and dead larvae were counted. Four replicates were conducted for each concentration. The lethal concentration (LC_{50}) for each extract was calculated using GraphPad Prism 6.0.

3 RESULTS

The chemical screening performed by thin-layer chromatography (*TLC*) and spot-tests revealed that the three evaluated species of *Parmotrema* exhibited secondary metabolite profiles consistent with ß-orcinol depsides and depsidones. Specifically, *P. latissimum* presented atranorin together with salazinic (and possibly consalazinic) acid; *P. mesotropum*

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showed atranorin and protocetraric acid; and *P. rubifaciens* contained atranorin plus norstictic, stictic and cryptostictic acids. These metabolite classes are widely recognized in lichen chemosystematics and contribute to the chemical diversity observed within the genus *Parmotrema* (Table 1).

Table 1

Chemical qualitative characterization of the Parmotrema species obtained extracts

Species	Chemistry (*TLC*)	Classes of metabolites
Parmotrema latissimum	Atranorin, salazinic and consalazinic acids	β-Orcinol Depsides and β-Orcinol Depsidones
Parmotrema mesotropum	Atranorin, and protocetraric acid	β-Orcinol Depsides and β-Orcinol Depsidones
Parmotrema rubifaciens	Atranorin, and norstictic, stictic, cryptostictic acids	β-Orcinol Depsides and β-Orcinol Depsidones

The three treatments (*Parmotrema latissimum*, *P. mesotropum*, and *P. rubifaciens*) evaluated through the larval immersion test against *Rhipicephalus microplus* (resistant Jaguar strain) exhibited a larval mortality rate of 0% under the tested conditions (Table 2). In all assays, larvae exposed to methanolic and hexanic extracts of the three lichen species remained alive after the 24-hour evaluation period.

These findings indicate that, at the concentrations and exposure parameters employed, the lichen extracts did not exert any detectable acaricidal activity on this multidrug-resistant tick lineage. This absence of efficacy suggests that either the secondary metabolites present in these *Parmotrema* extracts lack intrinsic toxicity to *R. microplus* larvae or that higher concentrations, alternative extraction methods, or different exposure times may be required to elicit a biological response.

Table 2

Larval mortality of Rhipicephalus microplus (Jaguar strain) exposed to lichen extracts at concentrations ranging from 0.4 to 2.0 mg/mL CL = lethal concentration; ME = methanolic extract; HE = hexanic extract.

Treatment (T)	Larval Mortality / CL_{50} (ME)	Larval Mortality / CL_{50} (HE)
T1 – *Parmotrema latissimum*	0%	0%
T2 – *Parmotrema mesotropum*	0%	0%
T3 – *Parmotrema rubifaciens*	0%	0%

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4 DISCUSSION

Certain environmental and methodological conditions may limit the effectiveness of biocontrol agents, including lichen-derived metabolites, against arthropods such as ticks. Factors such as prolonged exposure time required for bioactive compounds to induce target mortality, often longer than observed with conventional chemical acaricide, may reduce the apparent efficacy of natural products (Santi et al., 2011). Additionally, abiotic variables including temperature, humidity, and ultraviolet radiation can significantly affect the stability and activity of antimicrobial or antiparasitic agents in general. It is also possible that the specific chemical composition of the lichen extracts employed simply does not confer biological activity against *Rhipicephalus microplus* larvae under the tested conditions.

Although many lichens secondary metabolites (lichen acids) are known to exhibit a wide range of bioactivities, their effects are highly compound specific. For example, atranorin has been shown to inhibit herbivory and interfere with larval development in *Spodoptera ornithogalli* (Slansky, 1979); salazinic acid has demonstrated antitumor potential (Lira, 2021); derivatives of stictic acid have been associated with delayed larval development in *Spodoptera littoralis* (Giez et al., 1994); and protocetraric acid has exhibited antimicrobial properties (Tay et al., 2004). These documented activities highlight the biochemical diversity and ecological relevance of lichen metabolites, yet they also emphasize that not all compounds possess acaricidal properties, particularly against resistant tick lineages.

For *Parmotrema mesotropum*, the literature reports the presence of metabolites with remarkable biological properties. Mallavadhani et al. (2019) isolated major compounds such as methyl hematommate, methyl-2,4-dihydroxy-3,6-dimethylbenzoate, orcinol, and atranorin from *P. mesotropum*, and demonstrated that several chemically modified analogues exhibited potent anticancer effects against multiple human tumor cell lines. These findings highlight the chemical richness and pharmacological potential of this species. Nevertheless, in the present study, extracts of *P. mesotropum* showed no acaricidal activity against resistant *R. microplus* larvae, indicating that the bioactive profile of its metabolites does not translate into antiparasitic efficacy under the tested conditions. This contrast reinforces the idea that the biological activity of lichen compounds is strongly dependent on the target organism and mode of action.

Also, previous surveys in the Tocantinean region (southern Maranhão and northern Tocantins) demonstrated that local Parmeliaceae species synthesize a broad spectrum of lichen acids with documented antimicrobial, anti-inflammatory, antiproliferative, and other

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bioactivities, confirming the area as a promising hotspot for lichen bioprospecting (Dias et al., 2023).

5 CONCLUSION

The lichen extracts obtained from *Parmotrema latissimum*, *P. mesotropum*, and *P. rubifaciens*, each containing their characteristic secondary metabolites, such as atranorin, salazinic acid, protocetraric acid, and norstictic acid major, respectively, did not exhibit acaricidal activity against larvae of the resistant Jaguar strain of *Rhipicephalus microplus* under the concentrations and experimental conditions evaluated. The complete absence of larval mortality indicates that the metabolites present in these species, whether individually or as components of crude extracts, do not exert detectable biocontrol effects on this multidrug-resistant tick lineage when applied through the larval immersion test.

These results highlight the need for broader evaluations involving additional lichen taxa, other extraction strategies, and the isolated testing of specific compounds with documented or potential antiparasitic properties. Future studies should also consider assessing higher concentrations, extended exposure periods, and alternative bioassay formats to better characterize the acaricidal potential of lichen-derived metabolites. Such approaches may contribute to the identification of novel bioactive molecules suitable for sustainable tick management programs.

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