Veterinary Parasitology 340 (2025) 110620

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Chemical composition, acaricidal activity, and acetylcholinesterase inhibitory activity of two chemotypes of *Eugenia stictopetala* essential oil against *Rhipicephalus (Boophilus) microplus*

Antônia Fernanda Lopes da Silva [a], Júlia Karla de Albuquerque Melo Xavier [b], Marcos Bispo Pinheiro Camara [a], Cáritas de Jesus Silva Mendonça [c], Livio Martins Costa-Júnior [d], Wesley Douglas Ribeiro [e], Caio Pavão Tavares [e], Eloisa Helena de Aguiar Andrade [f], José Guilherme Soares Maia [g], Wallyson André dos Santos Bezerra [a], Alexandra Martins Santos Soares [a], Cláudia Quintino da Rocha [a,b,1,*]

[a] Graduate Program in Biodiversity and Biotechnology (Bionorte), Federal University of Maranhão, São Luís, MA 65080-805, Brazil
[b] Graduate Program in Chemistry (PPGQuim), Department of Chemistry, Federal University of Maranhão, São Luís, MA 65080-805, Brazil
[c] Graduate Program in Energy and Environment (PPGE), Federal University of Maranhão, São Luís, MA 65080-805, Brazil
[d] Graduate Program in Biotechnology (Renorbio), Federal University of Maranhão, São Luís, MA 65080-805, Brazil
[e] Parasite Control Laboratory, Department of Pathology, Federal University of Maranhão, São Luís, MA 65080-805, Brazil
[f] Adolpho Ducke Laboratory, Botany Coordination, Emílio Goeldi Museum of Pará, Belém, PA 66077-830, Brazil
[g] Graduate Program in Pharmaceutical Sciences, Federal University of Pará, Belém, PA 66075-110, Brazil

ARTICLE INFO

Keywords:
Citral
Essential oils
Myrtaceae
Myrcene
Natural acaricides
Enzyme inhibition

ABSTRACT

Rhipicephalus (Boophilus) microplus, an ectoparasite affecting livestock in tropical and subtropical regions, causes significant economic losses worldwide. In this context, effective control alternatives based on natural products are essential. This study evaluated the chemical composition, acaricidal activity, acute toxicity, and acetylcholinesterase (AChe) inhibition of essential oils (EO) from two chemotypes of *Eugenia stictopetala* (EOEs-1 and EOEs-2). Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed that both chemotypes were rich in monoterpene hydrocarbons (64.6 %). EOEs-1 was characterized mainly by myrcene (60.4 %) and citral (neral: 11.0 %; geranial: 15.1 %), whereas EOEs-2 contained myrcene (59.3 %), geraniol (20.0 %), and linalool (14.1 %) as its major constituents. Acaricidal activity was assessed by Larval Immersion Test (LIT) against *R. microplus* larvae, including the isolate compounds citral, myrcene, geraniol, and linalool. EOEs-1 exhibited the strongest acaricidal activity (LC_{50} of 1.798 mg/mL), with citral exhibiting similar potency (LC_{50}=1.639 mg/mL), while EOEs-2 showed an LC_{50} of 2.376 mg/mL. Additionally, both essential oils were capable of inhibiting the native acetylcholinesterase (AChE) of *R. microplus*, with EOEs-1 showing the strongest activity and emerging as a promising candidate for the control of this ectoparasite.

1. Introduction

Rhipicephalus (Boophilus) microplus is a common ectoparasite infesting cattle in tropical and subtropical regions, acting as a vector for diseases such as babesiosis and anaplasmosis, which results in considerable financial impacts on worldwide livestock farming (Grisi et al. 2014; Marques et al. 2020).

Currently, the control of *R. microplus* is primarily carried out using synthetic acaricides, such as organophosphates and carbamates, which act by inhibiting the enzyme acetylcholinesterase (AChE). This enzyme is responsible for cholinergic neurotransmission, and its inhibition can lead to convulsions and ultimately death in insects (Wu et al. 2013; Zhou and Xia, 2009).

However, the continuous and often indiscriminate use of these

* Corresponding author at: Graduate Program in Biodiversity and Biotechnology (Bionorte), Federal University of Maranhão, São Luís, MA 65080-805, Brazil.
E-mail address: rocha.claudia@ufma.br (C.Q. da Rocha).
[1] ORCID: 0000–0002-3578–1869

https://doi.org/10.1016/j.vetpar.2025.110620

Available online 2 October 2025

products has exerted significant selective pressure, leading to the accelerated emergence and spread of populations resistant to almost all synthetic chemical classes available on the market, including the identification of multi-resistant populations (Reck et al. 2014; Agwunobi et al. 2021).

In this context, exploring alternative treatment approaches, such as natural products, is necessary. These have significant advantages, including low toxicity to non-target organisms and rapid biodegradation of their residues (Singh et al. 2018). In this regard, many bioactive products, such as essential oils, are being extensively investigated to determine their efficacy against ticks and their mechanisms of action, constituting new control technologies (Pereira et al. 2022; Gonzaga et al. 2023).

Eugenia stictopetala Mart. ex DC. [syn. *Eugenia eschholtziana* O. Berg, *Eugenia martiusiana* DC., *Eugenia ochra* O. Berg, *Eugenia piauhiensis* O. Berg, *Eugenia roraimana* O. Berg, *Eugenia tapacumensis* O. Berg, among others] belongs to Myrtaceae and this family is known for its species diversity and importance in traditional medicine (World Flora Online (WFO), 2024). Also, *Eugenia* L. is a recognized fragrant genus with significant biological activities (Silveira et al. 2023).

The literature highlights the acaricidal effects in studies conducted with other *Eugenia* species essential oils against several arthropods. For example, the insecticidal and acaricidal properties of the *Eugenia uniflora* L. and *Eugenia caryophyllata* Thunb. against the maize weevils (*Sitophilus zeamais*), (Coitinho et al. 2011), dust mites (*Dermatophagoides farinae*), (Wu et al. 2010), and spotted spider mites (*Tetranychus urticae*) (Choi et al. 2004).

The oils of *Eugenia lutescens* Cambess. and *Eugenia langsdorffii* O. Berg. were evaluated and demonstrated a significant reduction in the oviposition of *Tetranychus urticae*, (Ribeiro et al. 2015) as the biocidal potential of oil from the leaves of *Eugenia pyriformis* Cambess. was reported against *R. microplus* (Medeiros et al. 2019).

Some studies of *E. stictopetala* have been previously published, such as its anticancer activity (Aranha et al. 2019) and leishmanicidal activity (Nunes et al. 2021). However, no previous work has reported the use of this oil for controlling the ectoparasite *R. microplus* or investigated the chemical variability among its specimes. Therefore, the present study aimed to evaluate the acaricidal, cytotoxic, and anticholinesterase activities of essential oils from two chemotypes of *E. stictopetala*.

2. Material and methods

2.1. Plant material

Two leaves samples of *E. stictopetala* (Myrtaceae), named EOEs-1 and EOEs-2, were collected in the city of São Bento, Maranhão state, Brazil, on September 9, 2023, under the coordinates 2º47'42.8" S and 45º00'11.0" W, respectively. The botanist Carlos Alberto S. da Silva identified plant materials, and the corresponding vouchers were deposited in the Museu Paraense Emílio Goeldi (MPEG), city of Belém, Pará state, Brazil, under the code number MG165442 as there were no apparent morphological differences. The specimens were registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen), Ministry of the Environment, Brazilian Government, under the number AEC4B8D.

2.2. Essential oil extraction

The leaves (300 g each) of the *E. stictopetala* specimens were air-dried at room temperature for two days and then subjected to hydrodistillation using a Clevenger-type apparatus for 3 h. Then, the distilled oils were dried over anhydrous sodium sulfate, and the yields were calculated based on the dry weight of the plant material. The moisture content of each sample was measured using an infrared moisture balance (Bel, Brazil, Model M214i) for the water loss measurement.

2.3. GC-MS analysis

The oil samples of *E. stictopetala* were analyzed on a GCMS-QP2010 Ultra system (Shimadzu Corporation, Tokyo, Japan) with an autoinjector (AOC-20i). The parameters of analysis were: A silica capillary column Rxi-5ms (30 m x 0.25 mm; 0.25 μm film thickness) (Restek Corporation, Bellefonte, PA, USA); injector temperature: 250 ºC; oven temperature programming: 60–240 ºC (3 ºC/min); helium as carrier gas, adjusted to a linear velocity of 36.5 cm/s (1.0 mL/min); splitless mode injection of 1 μL of sample (chemotype 1: 8,34 mg/mL; chemotype 2: 8,52 mg/mL); ionization by electronic impact at 70 eV; ionization source and transfer line temperatures at 200 and 250 ºC, respectively. The mass spectra were obtained by automatically scanning every 0.3 s, with mass fragments in the 35–400 *m/z* range. The retention index was calculated for all volatile components using a homologous series of C_8–C_{20} *n*-alkanes (Sigma-Aldrich, St. Louis, MI, USA), according to the linear equation of Van Den Dool and Kratz (1963). The oils constituents of oils were identified by comparing their retention indices and mass spectra (molecular mass and fragmentation pattern) with data stored in Adams Library (Adams, 2007).

2.4. Collection and maintenance of the ticks

Engorged females of *R. microplus* were collected after their natural detachment from artificially infested calves, washed in running water, dried on paper towels, and placed in Petri dishes. Then, the females were kept in the UFMA bioterium for oviposition, at 27 ± 1°C and relative humidity (RH) ≥ 80 %. At the end of oviposition, the eggs were transferred to 15 mL polyethylene tubes (Falcon type) for egg hatching under the same temperature and RH conditions. Larvae aged between 14 and 21 days were used in biological assays (Silva Lima et al. 2021). All procedures were approved through the Ethics Committee on Animal Experimentation of the Federal University of Maranhão, under protocol 23115.004153/2022–58.

2.5. Larval immersion test

The action of EOEs-1 and EOEs-2 oils, and their majority components citral, myrcene, linalool, and geraniol (purchased from Sigma-Aldrich, USA) in the ticks' larvae was evaluated using the larval immersion test as described by Klafke (2006). The treatment of the larvae with the oils and standards was carried out using the following concentrations: 5.0, 2.5, 1.25, 0.625, 0.3125, 0.156, 0.078, 0.039, and 0.0195 mg/mL. The dilutions were prepared in ethanol (1 %, v/v) and Triton X-100 (0.02 %, v/v). The control group was treated with the same diluent solution. Each solution (1 mL) was transferred to polypropylene microtubes (1.5 mL, Eppendorf type). The larvae (about 500) were immersed in each solution (for 10 min) and then dried on filter paper. The larvae (about 100) were placed in filter paper packets (8.5 cm×7.5 cm) and sealed with tubular plastic clips. The packages were kept in a BOD-type incubator at 27 ± 1 °C and RH of ≥ 80 % for 24 h. Larval mortality was determined from the total count of live and dead individuals. Larvae capable of moving their appendages but not walking were presumed dead. Larvae able to walk were given up alive. The test was performed in four independent assays, each with four replicates.

2.6. Evaluation of acute toxicity in vivo in the invertebrate model *Tenebrio molitor*

The larvae were obtained between the 10th and 12th larval stages (< 2 weeks old) from Biofábrica, São Luís, Ma, Brazil, a supplier specialized in the sale of invertebrate animals. They were kept in a dark environment, at room temperature, with free access to a standardized food substrate consisting of flaked cornmeal (Maratá®), breadcrumbs (Bumba Alimentos®), and fine oat flakes (Nestlé®) in a 2:2:2 ratio

(Navarro et al. 2019).

For the bioassay, larvae were selected based on size, weight, and color uniformity, without showing melanization, lesions, or alterations in motility (Navarro et al. 2019; Kavallieratos et al. 2021). The larvae were then divided into 10 groups (n = 10 larvae/group) and placed in sterile Petri dishes (Perfecta®) containing 4 g of the food substrate. The formation of the groups was determined based on the defined concentrations of the chemotypes for the study, namely: 1; 10; 100; and 1000 μg/mL-1, along with control (CTRL) groups for test evaluation and validation: CTRL Clean – control group that received no substance; CTRL Water – control group that received only water; CTRL C2H5OH – control group for solvent lethality (disinfection); CTRL Trauma – control group that received only the injection trauma; CTRL PBS – control group that received sterile PBS; CTRL DMSO 1 % – control group that received the diluent solvent of the chemotypes. The groups were then stored at room temperature (37ºC), protected from light, 24 h before the start of the assays (Navarro et al. 2019).

For the acute injection procedure of the chemotype concentrations, the larvae were initially subjected to paralysis by cooling (Navarro et al. 2019) using an artificial ice plate (Polar Técnica®, Brazil). Afterward, they were disinfected on the ventral portion with 70 % alcohol. Using an analytical syringe (Hamilton, Modified Microliter™), 10 μL of the different treatments were injected via the intrahemocelic route between the fourth and fifth metameres, on the ventral portion of the larvae (Navarro et al. 2019).

After the procedure, the larvae were transferred to Petri dishes (Perfecta®) designated for the respective groups, containing standardized food substrate, and incubated in the dark, in chambers with favorable temperature and humidity conditions. Survival was monitored daily for seven days, and larval death was assessed by response to touch, noting the lack of motility and the presence of melanization (Navarro et al. 2019; Lozoya-Pérez et al. 2021).

2.7. *In silico modeling and structural validation of Rhipicephalus microplus acetylcholinesterase (AChE)*

The amino acid sequence of RmAChE1 (GenBank accession number CAA11702.1) was retrieved from the NCBI database (www.ncbi.nlm.nih.gov). To identify an appropriate template for three-dimensional (3D) structure modeling, a sequence similarity search was conducted using the Protein Data Bank (PDB). The 3D model was constructed using the automated homology modeling software MODELLER version 10.2 (Webb and Sali, 2016). Among the 100 generated models, the most suitable conformation was selected based on the molpdf and DOPE scores. The final model was using the PROCHECK 3.0 server (Laskowski et al. 1993).

2.8. *Structure of compounds and molecular docking*

The structures of neral, geranial, myrcene, geraniol, linalool, and limonene were retrieved from the PubChem database (CIDs: 643779, 638011, 31253, 637566, 6549, and 440917, respectively) in mol2 format and subsequently optimized using the Avogadro software (Hanwell et al. 2012). To evaluate the binding affinity of these natural compounds to *R. microplus* AChE, molecular docking simulations were conducted at the enzyme's peripheral site using Molegro Virtual Docker 6.0 (MVD).

To identify the most appropriate docking protocol, the crystal structure of human acetylcholinesterase (AChE) complexed with the inhibitor 1YL (Dihydrotanshinone I) was retrieved from the Protein Data Bank (PDB ID: 4M0E – chain A; 2 Å resolution). Re-docking of 1YL into the binding site was performed, and protocols that resulted in a root-mean-square deviation (RMSD) of less than 2 Å from the original crystallographic pose were considered valid, in accordance with established criteria (Yusuf et al. 2008). The protocol that produced the lowest RMSD was selected for subsequent molecular docking simulations.

The molecular docking was carried out inside a virtual docking sphere with 15 Å and the following center coordinates: X: −17.19; Y: −42.33; Z: 25.74 Å. Ten independent docking runs were performed, and the results were reported as MolDock scores. The same parameters were applied to the molecular docking of selected natural products with *R. microplus* AChE. Notably, structural superimposition of the human and tick AChE models used in this study yielded a root-mean-square deviation (RMSD) of 0.181 Å, despite an amino acid sequence identity of 45.64 %. The best binding poses of the natural products with both AChEs were visualized and analyzed using PyMOL Molecular Graphics System v1.3 (http://www.pymol.org).

2.9. *Preparation of AChE extract and assessment of enzyme inhibition*

To extract AChE from *R. microplus*, the larvae were macerated in a mortar and pestle for 5 min in 100 mM sodium phosphate buffer (pH 7.0) containing 5 mM EDTA, 0.5 % (v/v) Triton X-100, and 5 μL/mL protease inhibitor mix (Sigma-Aldrich, St. Louis, MO, USA) at a ratio of 1:20 (larval weight to buffer volume). The homogenate was then centrifuged at 12,000 ×g for 30 min at 4 °C. The resulting supernatant was collected, stored at 4 °C, and used as the AChE source. Protein concentration was determined using bovine serum albumin (BSA) as the standard according to Bradford (1976).

AChE activity was measured following the method of Ellman et al. (1961), as modified by Li et al. (2005). Briefly, the reaction mixture contained 15 μL of AChE solution (0.8 μg protein), 95 μL of 50 mM sodium phosphate buffer (pH 7.5), and 100 μL of reaction solution composed of 0.24 mM acetylthiocholine iodide (Sigma-Aldrich) and 0.64 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (Sigma-Aldrich) prepared in the same buffer. The reaction was performed in a 96-well microplate for 30 min, with absorbance readings at 405 nm taken every 5 min using a Biochrom microplate reader.

Essential oils EOEs-1 and EOEs-2 were individually diluted in 50 mM sodium phosphate buffer (pH 7.5) to stock concentrations of 86 and 66 mg/mL, respectively. Working solutions at 2 mg/mL were subsequently prepared in the same buffer. AChE inhibition by the oils was assessed over a concentration range of 0.0625–1 mg/mL. For the inhibition assay, 15 μL of AChE solution was mixed with 95 μL of the essential oil solution and 100 μL of the reaction solution described above. Propoxur (Sigma-Aldrich) at 0.25 mM was used as a positive control. In negative controls, the compounds were replaced by phosphate buffer and methanol. All assays were performed in triplicate.

2.10. *Statistical analysis*

To estimate the 50 % lethal concentration (LC50), the concentrations of *E. stictopetala* oils were initially normalized and transformed into log (X), and non-linear regression analysis was performed using the GraphPad Prism 8.0.2 software (GraphPad Inc., San Diego, CA, USA). The significance of each comparison was determined when the calculated confidence intervals did not overlap. For the acute toxicity analysis, the data were plotted on Kaplan-Meier survival curves and analyzed using the Log-rank test. Statistical significance was defined as $p < 0.05$.

Additionally, for the enzyme inhibition tests, statistical analyses were conducted based on the mean values obtained, using ANOVA, followed by Tukey's test at a significance level of $p < 0.05$, using GraphPad Prism 8.0.2 software.

3. Results and discussion

3.1. *Yield and chemical composition*

In the present study, the essential oils of *E. stictopetala* leaves were obtained by hydrodistillation with a 1.9 % and 1.3 % yield for samples EOEs-1 and EOEs-2. The yield of *E. stictopetala* essential oil varies

according to the season and geographic origin of the species, as seen previously (Nunes et al. 2021; Dias et al. 2015; Veras et al. 2024).

Two specimens of *E. stictopetala*, collected in the Cerrado region of Chapada das Mesas National Park and in the city of Caxias, in the Maranhão state, presented different oil content, 0.4 %, and 2.1 %, respectively (Dias et al. 2015; Nunes et al. 2021). These data corroborate several studies carried out with some Myrtaceae species, indicating that the yield varies depending on the species and the local and collection season (Silva et al. 2017; Montalvan et al. 2019; Costa et al. 2020a). Thus, as different yields of essential oils, *Eugenia* species are characterized by intra- and interspecific chemical diversity (Costa et al. 2020a).

Using GC-MS analysis and spectra standard libraries, twenty-six constituents were identified in EOEs-1 and EOEs-2 samples, representing an average of 99.4 % of the total percentage identified. Monoterpene hydrocarbons predominated in both analyzed samples (EOEs-1, 64.6 % and EOEs-2, 64.6 %), followed by oxygenated monoterpenes (EOEs-1, 34.7 % and EOEs-2, 34.7 %). According to the analysis, two chemotypes have been defined in the chemical compositions of *E. stictopetala* oils. EOEs-1 was characterized by the presence of myrcene (60.4 %) and citral, a mixture of the stereoisomer's geranial (15.1 %) and neral (11.0 %), whereas EOEs-2 was by myrcene (59.3 %), geraniol (20.0 %), and linalool (14.1 %), described in Table 1.

According to the literature, sesquisterpenes predominate in *Eugenia* species, with germacrene-, humulane-, bisabolene-, and pinane-type dervivatives frequently reported (Silveira et al. 2023). Indeed, previous analysis of *E. stictopetala* from Maranhão and Amazonas described oils rich in γ-elemene, (*E*)-caryophyllene, germacrene D, bicyclogermacrene caryophyllene oxide, α-copaene, and α-pinene (Dias et al. 2015; Nunes et al. 2021; Aranha et al. 2019). In contrast, the present study revealed two chemotypes with a distinct predominance of monoterpenes, especially myrcene, accompanied by citral (geranial + neral) in EOE-1 and by geraniol and linalool in EOE-2. Notably, these oxygenated monoterpenes previously identified only in trace amounts in the *E. stictopetala* specimen from Caxias (MA, Brazil), previously reported (Nunes et al. 2021).

Although low levels of myrcene, linalool, and limonene have occasionally been reported in other specimens of *E. stictopetala* (Dias et al. 2015; Veras et al. 2024), the high concentrations observed in this study may indicate marked chemical polymorphism within the species, even among specimens collected in nearby regions. This variability is consistent with patterns documented in *Eugenia* and other *Myrtaceae*, where interspecific and intraspecific differences in oil composition are influenced by genetic, ecological, and geographic factors (Silveira et al. 2023; Contreras-Moreno, 2018; Jang et al. 2017).

Interestingly, the levels of myrcene found in our study exceed those commonly reported for other *Eugenia* species, where this monoterpene is usually restricted to minor proportions (5–10 %) (Apel et al., 2002; Moreno et al. 2007; Oliveros-Farjado and Pérez, 2011). Instead, they resemble the high myrcene contents characteristic of certain *Pimenta* species, such as *P. dioica* L. (6.0–26.76 %, from Mexico), *P. racemosa* (Mill.) JW Moore (29.97 %, from Jamaica), and *P. racemosa* var. *racemosa* (0.0–16.17 %, from Dominican Republic) (Contreras-Moreno, 2018). Similarly, the presence of citral (neral+geranial) in significant amounts aligns with profiles described in other Myrtaceae genera, including *P. pseudocaryophyllus* (Gomes) L.R. Landrum (Minas Gerais, Brazil), *Leptospermum petersonii* (Minas Gerais, Brazil), *Melaleuca teretifolia* Endl. (Australia) and *Calyptranthes sintenisii* Kiaersk. (Puerto Rico) (Paula et al. 2011; Tucker et al. 2001; Jang et al. 2017; Demuner et al. 2011). These parallels suggest that the biosynthesis of myrcene- and citral type monoterpenes may represent alternative metabolic routes within Myrtaceae, previously underrecognized in *Eugenia*.

Thus, our findings expand our chemical knowledge of *E. stictopetala*, revealing new chemotypes with a predominance of monoterpenes. This highlights the importance of considering intraspecific variability when evaluating the biological potential of essential oils in *Eugenia* and related genera.

002

Table 1
Composition of leaves essential oils from *Eugenia stictopetala*.

Constituents (%)	RI_C	RI_L	EOEs-1	EOEs-2
α-Pinene	933	932	0.3	0.4
β-Pinene	977	974	0.5	0.6
Myrcene	989	988	**60.4**	**59.3**
δ−3-Carene	1007	1008		0.1
p-Cymene	1024	1020	0.1	
Limonene	1029	1024	3.3	3.7
(*E*)-β-Ocimene	1045	1044		0.4
Linalool	1101	1095	1.0	**14.1**
allo-Ocimene	1129	1128		0.1
exo-Isocitral	1144	1140	0.6	
Isopulegol	1150	1145	0.1	
Citronellal	1153	1148	0.2	
(*Z*)-Isocitral	1162	1160	1.1	
p-Mentha−1,5-dien−8-ol	1173	1166	0.3	
(*E*)-Isocitral	1182	1177	2.5	0.1
γ-Terpineol	1198	1199	0.4	0.1
trans-Piperitol	1204	1207	0.1	
trans-Carveol	1209	1215	0.2	
Citronellol	1226	1223	0.3	
Nerol	1229	1227	0.8	0.1
Neral	1240	1235	**11.0**	0.1
Geraniol	1253	1249	0.9	**20.0**
Geranial	1271	1264	**15.1**	0.2
Methyl geranate	1325	1322	0.1	
(*E*)-Caryophyllene	1421	1417	0.1	
(*E*)-β-Farnesene	1459	1454	0.1	0.1
Monoterpene hydrocarbons			64.6	64.6
Oxygenated monoterpenes			34.7	34.7
Sesquiterpene hydrocarbons			0.2	0.1
Total (%)			99.5	99.4

RI_C = Calculated Retention index; RI_L = Literature Retention Index (based on Adams, 2007). Main constituents = bold.

3.2. Acaricidal activity

The chemotype oils (EOEs-1 and EOEs-2) of *E. stictopetala* showed acaricidal activity on tick larvae. EOEs-1 (LC_{50} 1.798 mg/mL) showed higher activity compared to EOEs-2 (LC_{50} 2.376 mg/mL) (non-IC overlap). Among the standard compounds tested, citral was the only one that showed acaricidal activity (LC_{50} 1.639 mg/mL). This value did not differ significantly from the 50 % lethal concentration (LC_{50}) obtained for the EOEs-1 chemotype (non-IC overlap). Myrcene, linalool, and geraniol showed a null value for larval mortality at the highest concentration (5.0 mg/mL) evaluated in this study. The assay data for each chemotype are listed in Table 2.

The literature describes a large number of essential oils with acaricidal properties, both *in vitro* and *in vivo*, which have the potential to attract, repel, and poison pests to varying degrees (Gonzaga et al. 2023; Bai et al. 2024). Myrtaceae species, particularly *Eugenia* spp, have shown that essential oils and their main components are highly toxic to *R. microplus* (Medeiros et al. 2019; Feder et al. 2019; Djebir et al. 2019).

Table 2
50 % Lethal concentration for the two oil chemotypes of *E. stictopetala* and some standard constituents.

Essential oil	LC_{50}	CI_{95}	Hill Slope	R^2
EOEs−1	1.798	1.659–1.947	3.124	0.938
EOEs−2	2.376	2.029–2.765	1.448	0.854
Citral (geranial + neral)	1.639	1.503–1.752	2.579	0.972
Myrcene	> 5000	-	-	-
Linalool	> 5000	-	-	-
Geraniol	> 5000	-	-	-

LC_{50}: Lethal concentration (mg/mL) for 50 % of individuals; CI_{95}: Confidence interval of 95 %; Hill Slope: Slope of the dose-response curve; R^2: Regression correlation coefficient.

In this study, the main essential oils components tested were myrcene and citral (neral and geranial), identified in the EOEs-1 chemotype, and myrcene, geraniol, and linalool in the EOEs-2 chemotype. The highest acaricidal activity was observed for chemotype EOEs-1, possibly related to the presence of citral, which is absent in chemotype EOEs-2. When evaluated alone, citral presented an LC_{50} of 1.639 mg/mL on *R. microplus* larvae, a close value to that given by the EOEs-1 chemotype oil (LC_{50} 1.798 mg/mL).

In a previous study, citral showed an LC_{50} of 1.13 mg/mL on an *R. microplus* population larvae, said to be resistant to organophosphates, synthetic pyrethroids, phenylpyrazoles, amidines, macrocyclic lactones, and benzoylphenyl compounds (Cardoso et al. 2020). The same study also determined an LC_{50} of 0.38 mg/mL for a population of larvae sensitive to the same synthetic acaricides.

The acaricidal activity of citral was previously reported in a study using two chemotype oils of *Lippia alba* (Mill.) N.E. Brown from the Active Germplasm Bank of the Federal University of Sergipe. In this study, the genotypes of the carvone chemotype included LA-13 (Fortaleza, CE) and LA-57 (Bahia), while those of the citral chemotype were represented by LA-10 and LA-44 (Brasília, DF). The citral-rich chemotypes, composed predominantly of geranial (46.2 % in LA-10 and 44.2 % in LA-44) and neral (33.5 % and 31.1 %, respectively), showed greater larvicidal activity, with LC_{50} values of 8.8 mg/mL and 10.9 mg/mL (Peixoto et al. 2015).

In the present study, linalool did not show acaricidal activity up to the maximum concentration of 5 mg/mL, demonstrating its relative ineffectiveness for controlling *R. microplus* larvae. Previously, the acaricidal activity of this oxygenated monoterpene on the *R. microplus* larvae was evaluated, exhibiting meager mortality rates for the groups treated with linalool, reaching only a 14.5 % value at the highest concentration (20.0 µl/mL) (Senra et al. 2013). In the same way, geraniol did not show acaricidal activity up to the maximum concentration of 5 mg/mL. However, in a study conducted on larvae of 10 different populations of *R. microplus*, geraniol presented an LC_{50} ranging from 0.36 % to 1.12 %, a significant concentration when considering the possibility of incorporating this molecule into a product for controlling cattle ticks, given the financial cost (Singh et al. 2018).

This study also evaluated a botanical product on *R. microplus* larvae, the Essentria® IC-3 concentrate, containing rosemary oil (10 %), geraniol (5 %), and peppermint oil (2 %), obtaining LC_{50} values ranging from 0.597 % to 1.674 %. On the other hand, the Essentria® IC-3 concentrate was evaluated *in vitro* at a concentration of 6.25 %, showing 100 % mortality in larvae and 94 % mortality in engorged females of *R. Microplus* (Klafke et al. 2021). In *in vivo* tests, the Essentria® IC-3 concentrate showed efficacy of only 70 %, and the same authors do not recommend it as a standalone method for controlling *R. microplus*.

Regarding myrcene, the main constituent of *E. stictopetala* oils, zero mortality of *R. microplus* larvae was observed at the highest concentration (5.0 mg/mL). This result suggests that the acaricidal activity observed in the two chemotypes evaluated (EOEs-1 and EOEs-2) may not be directly related to the isolated action of myrcene, but to possible synergistic interactions between the various constituents present in the essential oils, with emphasis on citral, which has been associated with acaricidal effects in previous studies (Peixoto et al. 2015).

It is important to note that there are no reports in the literature on the acaricidal effect of myrcene on ticks. However, this molecule shows a range of biological activities, making it a compound of interest both for traditional medicine and the pharmaceutical industry. Among these activities, the potent protection of the gastric mucosa stands out, involving the activation of antioxidant substances such as glutathione and nitric oxide, anticancer activity in various cancer cells, and antitrypanosomal activity (Bonamin et al. 2014; Bai and Tang. 2020; Pincigher et al. 2023; Medbouhi et al. 2019).

3.3. Acute toxicity of chemotypes 1 and 2 in T. molitor

In the search for new naturally derived products, the evaluation of the toxicity of compounds becomes necessary to ensure safety and feasibility during use. In this context, invertebrate models, such as insect larvae, are employed due to their rapid reproduction, low maintenance costs, and their role as experimental models that contribute to reducing costs and the number of rodents used in laboratory tests, following the principles of the 3Rs (Reduce, Replace, and Refine (Brai et al. 2023).

In this study, using *T. molitor* larvae, the tested concentrations exhibited different effects on *T. molitor* survival for both EOEs-1 and EOEs-2. Statistical analysis indicated no significant differences between the groups of the two chemotypes ($p > 0.005$). However, when comparing the chemotypes, a decrease in survival was observed with increasing concentration in both cases. Notably, for EOEs-2, the reduction was more pronounced at concentrations of 10 µg and 100 µg, whereas the 1000 µg concentration led to accelerated mortality compared to EOEs-1. This suggests a potential variation in toxicity in this assay, as EOEs-2 at 1000 µg induced the mortality of three larvae within the first 24 h, whereas EOEs-1 caused the death of only one larva at the same concentration (Fig. 1).

The observed variation in toxicity may be related to differences in the composition of the chemotypes, as the concentration of citral (neral + geranial) in EOEs-1 is lower compared to EOEs-2. The evaluation of essential oils of *Menta spicata, Cymbopogon flexuosus, Eugenia caryophyllus*, *Thymus vulgaris*, Lavandula x hybrida and *Eucalyptus globulus* on the behavior of *T. molitor* revealed that the essential oil of *M. spicata* presented a carvone content of 78.01 %, which was considered the most effective, whereas the oil of *E. caryophyllus* exhibited moderate effects, likely due to the activity of eugenol (75.86 %). In contrast, *E. globulus* showed no significant effects. Additionally, these authors tested analytical standards of myrcene, limonene, and γ-terpinene, none of which demonstrated any activity on larval behavior (Bumbulyte et al. 2023).

The study by Costa et al. (2020b) evaluated the toxicity and repellent effects of essential oils from *Ocimum basilicum* cultivars and the main monoterpenes in their composition against stored grain pests. The primary compounds analyzed were linalool, (*E*)-methyl cinnamate, and citral. Regarding toxicity, (*E*)-methyl cinnamate was identified as the most toxic compound, whereas citral exhibited the most significant repellent effect.

In this study, higher concentrations caused a significant reduction in larval survival. The results showed agreement with the controls tested, indicating that the concentrations tested may be below the toxicity limit, which makes the chemotypes safe at lower concentrations.

3.4. Modelling of the three-dimensional structure of R. microplus acetylcholinesterase (AChE)

The human AChE structure (PDB ID: 4M0E, Chain A) was selected as the template for homology modelling of *R. microplus* AChE. This choice was based on its structural quality, 45.65 % sequence identity, and 89 % sequence coverage with RmAChE1. The best homology model generated using MODELLER v.10.2 showed a molpdf score of 5482.30 and a DOPE score of −64004.38. The stereochemical quality of the model was confirmed by Ramachandran plot analysis, which showed that 86.4 % of residues fall within the most favored regions (Supplementary Figure 1).

3.5. Re-docking and molecular docking

Re-docking protocol, which employed the MolDock Score [GRID] as scoring function and the iterated simplex search algorithm, resulted in an RMSD of 0.169 Å and was therefore selected for the molecular docking simulations (Supplementary Figure 2). *In silico* molecular docking results predicted that the tested compounds have binding affinity toward of the *R. microplus* AChE, with more negative MolDock

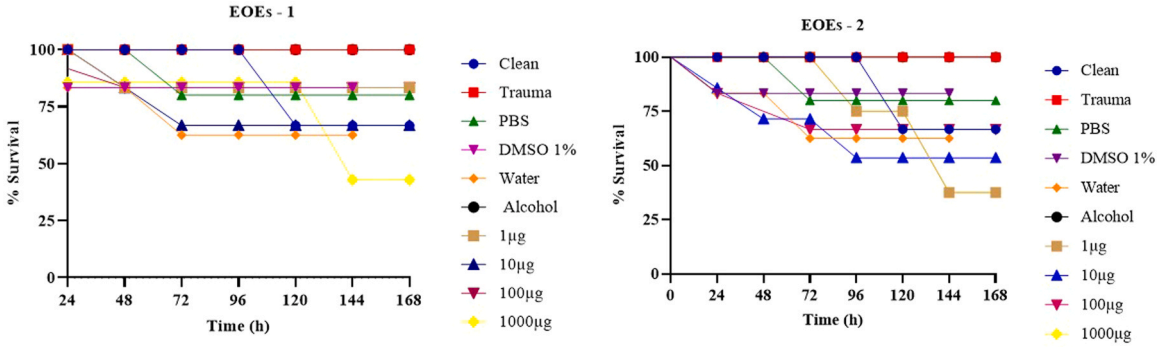
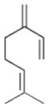
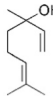


Fig. 1. Analysis of the survival of *Tenebrio molitor* under the action of different concentrations of two chemotypes of *E. stictopetala*. EOEs – 1: Chemotype 1; EOEs – 2: Chemotype 2; Clean: Clean Control; Trauma: Trauma Control; PBS: PBS Control; DMSO 1 %: DMSO Control; Water: Water Control; Alcohol: Alcohol Control.

scores indicating stronger predicted affinity (Table 3). Interaction analysis revealed that these compounds engage with several amino acid residues of the *R. microplus* AChE active site (Fig. 2). Docking simulations were performed using neral, geranial, myrcene, geraniol, linalool, and limonene against the Rhipicephalus microplus AChE model to predict their optimal binding conformations and estimate binding energies (Malak et al. 2023). Lower MolDock scores correspond to higher predicted binding affinities (Table 3, Fig. 2).

In this study, myrcene, geraniol, and linalool were predicted to interact with several amino acid residues of *R. microplus* AChE, including Asn336 (Fig. 2). The importance of Asn336 as a binding site has also been emphasized in previous studies involving natural compounds such as rutin and quercetin, which not only form hydrogen bonds with this residue but also contribute to the stabilization of enzyme–ligand complexes. These findings indicate a key role for Asn336 in ligand recognition and complex stabilization (Bustos-Baena et al. 2024). In contrast, neral, which achieved the most favorable Moldock score, and limonene interact with Phe385, among other residues. Likewise, chlorogenic acid is predicted to interact with amino acid residues in *R. microplus* AChE, including Phe385, further supporting the relevance of this residue in the stabilization of enzyme–ligand complexes (Bustos-Baena et al. 2024).

Table 3
MolDock scores of predominant monoterpenes in *E. stictopetala* chemotypes 1 and 2 against of the *R. microplus* AChE.

Compounds	PubChem CID	Molecular Structure	Moldock Score
Neral	643779		−97.650
Geranial	638011		−79.236
Myrcene	31253		−68.905
Geraniol	637566		−67.448
Linalool	6549		−66.286
Limonene	440917		−57.886

3.6. Inhibition of R. microplus acetylcholinesterase (AChE)

Experimental assays demonstrated that EOEs-1 and EOEs-2 inhibited AChE activity in *R microplus* to varying degrees. The highest level of inhibition — 50.41 % — was observed for EOEs-2, while for EOEs-1 an inhibition of 45.02 % was observed, both at a concentration of 1 mg/mL (Fig. 3).

The anticholinesterase activity of two chemotypes of *Myrcia splendens* essential oil was investigated, and differences in their inhibitory efficacy against AChE were identified (Gonçalves et al. 2025). Chemotype B, characterized by a high content of carotol (18.08 %), exhibited significant AChE inhibition (IC_{50} = 0.14 mg/mL), whereas chemotype A, rich in (2*E*,6*E*)-methyl farnesoate (42.64 %), showed low inhibitory activity at the same concentrations. These findings suggest that the biological activity of chemotype A may be associated with alternative molecular targets rather than AChE inhibition.

Another relevant finding of the same study was that chemotype A, despite its low anticholinesterase activity, demonstrated acaricidal effects in bioassays. This result corroborates the findings of Cardoso et al. (2020), who observed that certain terpenes, such as citral, exert acaricidal effects without significantly inhibiting AChE. Taken together, these findings indicate that other molecular targets may contribute to the observed activity, and therefore, the elucidation of these alternative mechanisms becomes decisive for the development of new strategies against resistance to conventional acaricides.

The observed *in vitro* inhibition of native *R. microplus* AChE by essential oils—despite requiring relatively high concentrations—suggests the presence of constituent(s) capable of interfering with enzymatic function. These active compounds may be present in low abundance, thus requiring higher doses of the crude oil to elicit measurable effects. Alternatively, the observed inhibition may result from additive or synergistic effects among multiple minor constituents. These findings highlight the need for further phytochemical characterization and fractionation of the oils to isolate the most active inhibitory component(s), which could possess greater potency in purified form.

4. Conclusions

Although previous studies on *E. stictopetala* have reported the predominance of sesquiterpenes as major constituents, the present study identified a distinct chemical profile, characterized by the predominance of monoterpenes, highlighting myrcene, citral (neral and geranial), geraniol and linalool, little described as major compounds for this species. The present findings indicate that chemotype 1 (EOEs-1) exhibits greater acaricidal activity than chemotype 2 (EOEs-2). The observed results may be associated with the presence of citral (neral and

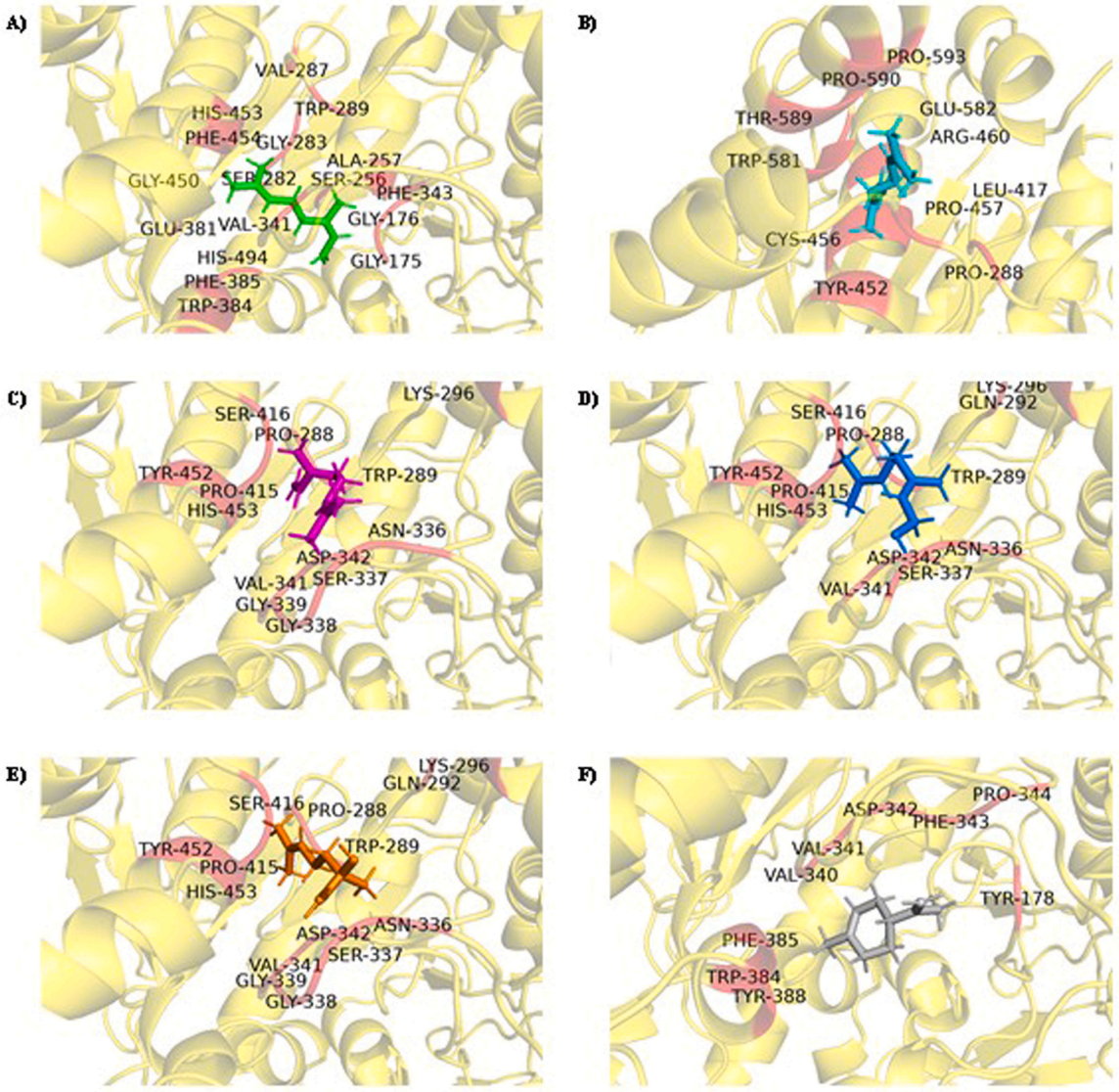


Fig. 2. Cartoon representations of *R. microplus* AChE (yellow) in complex with: A) Neral, B) Geranial, C) Myrcene, D) Geraniol, E) Linalool, and F) Limonene. Ligands are shown as sticks; AChE residues within 3.5 Å of the ligands are highlighted in red.Fonte: Images generated using PyMOL v1.3.

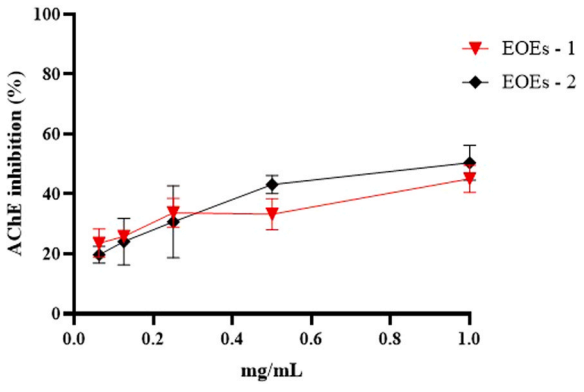


Fig. 3. Effect of different concentrations of essential oils (mg/mL) on the acetylcholinesterase activity of *R. microplus* expressed as percentage of inhibition (%). EOEs-1: Chemotype1. EOEs-2: Chemotype 2.

geranial) in chemotype 1, which may act synergistically with other main constituents and contribute to the acaricidal effect against *R. microplus* larvae. Given the absence of toxicity at low concentrations, this chemotype represents a promising candidate for the development of acaricides targeting cattle ticks. The next steps of this study will be to evaluate the effect of *E. stictopetala* oils on engorged females for the development of a formulation based on natural products with acaricidal potential.

CRediT authorship contribution statement

Livio Martins Costa-Júnior: Writing – review & editing, Funding acquisition. **Wesley Douglas Ribeiro:** Writing – original draft, Methodology, Investigation. **Marcos Bispo Pinheiro Camara:** Methodology. **Cáritas de Jesus Silva Mendonça:** Methodology. **Cláudia Quintino da Rocha:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Antônia Fernanda Lopes da Silva:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Júlia Karla de Albuquerque Melo Xavier:** Writing – original draft, Methodology.

Alexandra Martins Santos Soares: Writing – review & editing, Funding acquisition, Formal analysis. **José Guilherme Soares Maia:** Writing – review & editing, Formal analysis. **Wallyson André dos Santos Bezerra:** Writing – original draft, Methodology, Investigation. **Caio Pavão Tavares:** Writing – review & editing, Investigation, Formal analysis. **Eloisa Helena de Aguiar Andrade:** Writing – review & editing, Investigation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Claudia Quintino da Rocha reports financial support was provided by Federal University of Maranhao. CLAUDIA QUINTINO DA ROCHA reports a relationship with Federal University of Maranhao that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank the Emílio Goeldi Museum for its contribution to the analysis and botanical identification of the specimen. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001, PROCAD-AM [88887.472618/2019–00 (C.Q.R)], PDPG-PosDoc [88887.691481/2022–00 (C.Q.R,)], PPBio/CNPQ [441189/2023–7 (C.Q.R)] and FAPEMA [INFRA-02263/21 (C.Q.R)].

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2025.110620.

References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry, fivth ed. Texensis Publishing, Gruver, TX, USA.

Agwunobi, D.O., Yu, Z., Liu, J., 2021. A retrospective review on ixodid tick resistance against synthetic acaricides: implications and perspectives for future resistance prevention and mitigation. Pestic. Biochem. Physiol. 173, 104776. https://doi.org/10.1016/j.pestbp.2021.104776.

Apel, M.A., Limberger, R.P., Sobral, M., Henriques, A.T., Ntalani, H., Vérin, P., Menut, C., Bessiere, J.M., 2002. Chemical composition of the essential oils from Southern Brazilian *Eugenia* species. Part III. J. Essent. Oil Res. 14, 259–262. https://doi.org/10.1080/10412905.2002.9699847.

Aranha, E.S.P., Azevedo, S.G., Reis, G.G., Lima, E.S., Machado, M.B., Vasconcellos, M.C., 2019. Essential oils from *Eugenia* spp.: in vitro antiproliferative potential with inhibitory action of metalloproteinases. Ind. Crops Prod. 141, 111736. https://doi.org/10.1016/j.indcrop.2019.111736.

Bai, L., Gao, Z., Xu, X., Lv, W., Wang, Y., Dong, K., Yu, Z., Yang, X., 2024. The acaricidal activity and enzymatic targets of the essential oils of *Cinnamomum cassia* and *Cinnamomum camphora* and their major components against *Haemaphysalis longicornis* (Acari: Ixodidae). Ind. Crops Prod. 209, 117967. https://doi.org/10.1016/j.indcrop.2023.117967.

Bai, X., Tang, J., 2020. Myrcene exhibits antitumor activity against lung cancer cells by inducing oxidative stress and apoptosis mechanisms. Nat. Prod. Commun. 15, 1–7. https://doi.org/10.1177/1934578X20961189.

Bonamin, F., Moraes, T.M., Santos, R.C., Kushima, H., Faria, F.M., Silva, M.A., Hiruma-Lima, C.A., 2014. The effect of a minor constituent of essential oil from *Citrus aurantium*: the role of β-myrcene in preventing peptic ulcer disease. Chem. Biol. Inter. 212, 11–19. https://doi.org/10.1016/j.cbi.2014.01.009.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Brai, A., Poggialini, F., Vagaggini, C., Pasqualini, C., Simoni, S., Francardi, V., Dreassi, E., 2023. *Tenebrio molitor* as a simple and cheap preclinical pharmacokinetic and toxicity model. Int. J. Mol. Sci. 24, 2296. https://doi.org/10.3390/ijms24032296.

Bumbulytė, G., Būdienė, J., Būda, V., 2023. Essential oils and their components control behaviour of yellow mealworm (*Tenebrio molitor*) larvae. Insects 14, 636. https://doi.org/10.3390/insects14070636.

Bustos-Baena, A.S., Bravo-Ramos, J.L., Romero-Salas, D., Sánchez-Montes, S., Ortiz-Carbajal, L.A., Sánchez-Otero, M.G., 2024. *In vitro* and *in silico* studies of the acaricidal and anticholinesterase activities of *Randia aculeata* seeds against the Southern cattle tick *Rhipicephalus (boophilus) microplus*. Rev. Bras. Parasitol. Vet. 33, 1–13. https://doi.org/10.1590/S1984-29612024021.

Cardoso, A.S., Santos, E.G.G., Silva Lima, A., Temeyer, K.B., Perez Leon, A.A., Costa-Junior, L.M., Soares, A.M.S., 2020. Terpenes on *Rhipicephalus (boophilus) microplus*: acaricidal activity and acetylcholinesterase inhibition. Vet. Parasitol. 280, 109090. https://doi.org/10.1016/j.vetpar.2020.109090.

Choi, W.I., Lee, S.G., Park, H.M., Ahn, Y.J., 2004. Toxicity of plant essential oils to *Tetranychus urticae* (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae). J. Econ. Entomol. 97, 553–558. https://doi.org/10.1093/jee/97.2.553.

Coitinho, R.L.B.D., Oliveira, J.V., Junior, Gondim, Câmara CAG, M.G.C., 2011. Toxicidade por fumigação, contato e ingestão de óleos essenciais para *Sitophilus zeamais* motschulsky, 1885 (Coleoptera: Curculionidae). Cienc. Agrotec. 35, 172–178. https://doi.org/10.1590/S1413-70542011000100022.

Contreras-Moreno, B.Z., 2018. Chemical composition of essential oil of genus *Pimenta* (Myrtaceae). In: El-Shemy, H (Ed.), *Potential of Essential Oils*. IntechOpen Limited, London, UK, p. 21.

Costa, J.S., Barroso, A.S., Mourão, R.H.V., Silva, J.K.R., Maia, J.G.S., Figueiredo, P.L.B., 2020. Seasonal and antioxidant evaluation of essential oil from *Eugenia uniflora* L., curzerene-rich, thermally produced *in situ*. Biomolecules 10, 328. https://doi.org/10.3390/biom10020328.

Costa, A.S., Arrigoni-Blank, M.F., Bacci, L., Blank, A.F., Alves, P.B., Peixoto, M.G., Santos, A.C.C., 2020. Toxicity of the essential oil of basil cultivars and hybrids and its repellent effect on stored grain pests. Biosci. J. 36, 341–352. https://doi.org/10.14393/BJ-v36n2a2020-42266.

Demuner, A.J., Barbosa, L.C.A., Magalhães, C.G., Silva, J.C., Maltha, C.R.A., Pinheiro, A. L., 2011. Seasonal variation in the chemical composition and antimicrobial activity of volatile oils of three species of *Leptospermum* (Myrtaceae) grown in Brazil. Molecules 16, 1181–1191. https://doi.org/10.3390/molecules16021181.

Dias, C.N., Alves, L.P.L., Rodrigues, K.A.F., Brito, M.C.A., Rosa, C.D.S., Amaral, F.M.M., Monteiro, O.S., Andrade, E.H.A., Maia, J.G.S., Moraes, D.F.C., 2015. Chemical composition and larvicidal activity of essential oils extracted from Brazilian legal Amazon plants against *Aedes aegypti* L. (Diptera: Culicidae). Evid. Based Complement. Altern. Med. 2015, 490765. https://doi.org/10.1155/2015/490765.

Djebir, S., Ksouri, S., Trigui, M., Tounsi, S., Boumaaza, A., Hadef, Y., Benakhla, Y.A., 2019. Chemical composition and acaricidal activity of the essential oils of some plant species of lamiaceae and myrtaceae against the vector of tropical bovine theileriosis: H*yalomma scupense* (syn. H*yalomma detritum*). Biomed. Res. Int. 2019, 7805467. https://doi.org/10.1155/2019/7805467.

Ellman, G.L., Courtney, K.D., Andres Jr, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharm. 7, 88–95. https://doi.org/10.1016/0006-2952(61)90145-9.

Feder, D., Gonzalez, M.S., Mello, C.B., Santos, M.G., Rocha, L., Kelecom, A., Folly, E., 2019. Exploring the insecticide and acaricide potential of development regulators obtained from restinga vegetation from Brazil. An. Acad. Bras. Ciênc. 91, e20180381. https://doi.org/10.1590/0001-3765201920180381.

Gonçalves, H.S.L., Nascimento Silva, A., Botura, M.B., Carreira, D.S.S., Uzêda, R.S., Lucchese, A.M., 2025. Acaricidal activity of *Myrcia splendens* essential oils on *Rhipicephalus microplus* larvae. Vet. Parasitol., 110491 https://doi.org/10.1016/j.vetpar.2025.110491.

Gonzaga, B.C.F., Barrozo, M.M., Coutinho, A.L., Sousa, L.J.M.P., Vale, F.L., Marreto, L., Marchesini, P., Rodrigues, D.C., Souza, E.D.F., Sabatini, G.A., Costa-Júnior, L.M., Ferreira, L.L., Lopes, W.D.Z., Monteiro, C., 2023. Essential oils and isolated compounds for tick control: advances beyond the laboratory. Parasites Vectors 16, 415. https://doi.org/10.1186/s13071-023-05969-w.

Grisi, L., Leite, R.C., Martins, J.R.D.S., Barros, A.T.M., Andreotti, R., Cançado, P.H.D., León, A.A.P., Pereira, J.B., Villela, H.S., 2014. Reassessment of the potential economic impact of cattle parasites in Brazil. Rev. Bras. Parasitol. Vet. 23, 150–156. https://doi.org/10.1590/S1984-29612014042.

Hanwell, M.D., Curtis, D.E., Lonie, D.C., Vandermeersch, T., Zurek, E., Hutchison, G.R., 2012. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. J. Chemin. 4, 17. https://doi.org/10.1186/1758-2946-4-17.

Jang, M., Kim, J., Yoon, K.A., Lee, S.H., Park, C.G., 2017. Biological activity of myrtaceae plant essential oils and their major components against *Drosophila suzukii* (Diptera: Drosophilidae). Pest Manag. Sci. 73, 404–409. https://doi.org/10.1002/ps.4430.

Kavallieratos, N.G., Skourti, A., Nika, E.P., Ntalaka, C.T., Boukouvala, M.C., Bonacucina, G., Cespi, M., Petrelli, R., Cappellacci, L., Maggi, F., Benelli, G., Canale, A., 2021. Isofuranodiene-based nanoemulsion: larvicidal and adulticidal activity against tenebrionid beetles attacking stored wheat. J. Stored Prod. Res. 93, 101859. https://doi.org/10.1016/j.jspr.2021.101859.

Klafke, G.M., Thomas, D.B., Miller, R.J., de León, A.A.P., 2021. Efficacy of a water-based botanical acaricide formulation applied in portable spray box against the Southern cattle tick, *Rhipicephalus (boophilus) microplus* (Acari: Ixodidae), infesting cattle. Ticks Tick. Borne Dis. 12 (4), 101721. https://doi.org/10.1016/j.ttbdis.2021.101721.

Laskowski, R.A., MacArthur, M.W., Moss, D.S., Thornton, J.M., 1993. PROCHECK - a program to check the stereochemical quality of protein structures. J. Appl. Crystallogr 26, 283–291. https://doi.org/10.1107/S0021889892009944.

Li, A.Y., Pruett, J.H., Davey, R.B., George, J.E., 2005. Toxicological and biochemical characterization of coumaphos resistance in the san roman strain of *Boophilus* microplus (Acari: Ixodidae). Pestic. Biochem. Physiol. 81, 145–153. https://doi.org/10.1016/j.pestbp.2004.12.002.

Lozoya-Pérez, N.E., García-Carnero, L.C., Martínez-Álvarez, J.A., Martínez-Duncker, I., Mora-Montes, H.M., 2021. *Tenebrio molitor* as an alternative model to analyze the *sporothrix* species virulence. Infect. Drug Resist 14, 2059–2072. https://doi.org/10.2147/IDR.S312553.

Malak, N., Alotaibi, B.S., Khan, A., Khan, A., Ullah, S., Nasreen, N., Chen, C.C., 2023. Density functional theory calculations and molecular docking analyses of flavonoids for their possible application against the acetylcholinesterase and triose-phosphate isomerase proteins of *R*hipicephalus microplus. Molecules 28, 3606. https://doi.org/10.3390/molecules28083606.

Marques, R., Krüger, R.F., Peterson, A.T., Melo, L.F., Vicenzi, N., Jiménez-García, D., 2020. Climate change implications for the distribution of the babesiosis and anaplasmosis tick vector, *Rhipicephalus (boophilus) microplus*. Vet. Res. 51, 1–10. https://doi.org/10.1186/s13567-020-00802-z.

Medbouhi, A., Benbelaïd, F., Djabou, N., Beaufay, C., Bendahou, M., Quetin-Leclercq, J., Tintaru, A., Costa, J., Muselli, A., 2019. Essential oil of Algerian *Eryngium campestre*: chemical variability and evaluation of biological activities. Molecules 24, 2575. https://doi.org/10.3390/molecules24142575.

Medeiros, J.P., Bortollucci, W.C., Silva, E.S., Oliveira, H.L., Campo, C.F., Gonçalves, J.E., Piau-Junior, R., Gazim, Z.C., 2019. Biocidal potential of *Eugenia pyriformis* essential oil in the control of *Rhipicephalus (boophilus) microplus* in the free-living cycle. Pesqui. Vet. Bras. 39, 879–888. https://doi.org/10.1590/1678-5150-PVB-6434.

Montalván, M., Peñafiel, M.A., Ramírez, J., Cumbicus, N., Bec, N., Larroque, C., Bicchi, C., Gilardoni, G., 2019. Chemical composition, enantiomeric distribution, and sensory evaluation of the essential oils distilled from the Ecuadorian species *Myrcianthes myrsinoides* (Kunth) grifo and *Myrcia mollis* (Kunth) DC. (Myrtaceae). Plants 8 (11), 511. https://doi.org/10.3390/plants8110511.

Moreno, P.R.H., Lima, M.E.L., Sobral, M., Young, M.C.M., Cordeiro, I., Apel, M.A., Limberger, R.P., Henriques, A.T., 2007. Essential oil composition of fruit colour varieties of *Eugenia brasiliensis* lam. Sci. Agric. 64, 428–432. https://doi.org/10.1590/S0103-90162007000400014.

Navarro, M.O.P., Simionato, A.S., Pérez, J.C.B., Barazetti, A.R., Emiliano, J., Niekawa, E. T.G., Andreata, M.F.L., Modolon, F., Dealis, M.L., Araújo, E.J.A., Carlos, T.M., Scarpelim, O.J., Silva, D.B., Chryssafidis, A.L., Bruheim, P., Andrade, G., 2019. Fluopsin c for treating multidrug-resistant infections: in vitro activity against clinically important strains and in vivo efficacy against carbapenemase-producing *Klebsiella pneumoniae*. Front. Microbiol. 10, 2431. https://doi.org/10.3389/fmicb.2019.02431.

Nunes, T.M.L., Costa, L.H., Sousa, J.M.S., Sousa, V.M.R., Rodrigues, R.R.L., Val, M.C.A., Pereira, A.C.T.C., Ferreira, G.P., Silva, M.V., Costa, J.M.A.R., Véras, L.M.C., Diniz, R. C., Rodrigues, K.A.F., 2021. *Eugenia piauhiensis* vell. Aff. essential oil and γ-elemene its major constituent exhibit antileishmanial activity, promoting cell membrane damage and *in vitro* immunomodulation. Chem. Biol. Inter. 339, 109429. https://doi.org/10.1016/j.cbi.2021.109429.

Oliveros-Fajardo, A., Pérez, A.L.M., 2011. Composition of arazá (*eugenia stipitata* McVaugh) seed essential oil. Momentos Cienc 8 (2).

Paula, J.A., Ferri, J.H., Bara, M.T.F., Tresvenzol, L.M., Sá, F.A., Paula, J.R., 2011. Infraspecific chemical variability in the essential oils of *Pimenta pseudocaryophyllus* (Gomes) L.R. Landrum (Myrtaceae). Biochem. Syst. Ecol. 39, 643–650. https://doi.org/10.1016/j.bse.2011.05.013.

Peixoto, M.G., Costa-Júnior, L.M., Blank, L.M., Lima, A.S., Menezes, T.S.A., Santos, D.A., Alves, P.B., Cavalcanti, S.C.H., Bacci, L., Arrigoni-Blank, M.F., 2015. Acaricidal activity of essential oils from *Lippia alba* genotypes and its major components carvone, limonene, and citral against *Rhipicephalus microplus*. Vet. Parasitol. 210, 118–122. https://doi.org/10.1016/j.vetpar.2015.03.010.

Pereira, J.R., Silva, S.M.P., Marques, M.O.M., 2022. Efficacy of essential oil of *Lippia sidoides* (Verbenaceae) for controlling the cattle tick *Rhipicephalus (boophilus) microplus* on naturally parasitized animals under field conditions. Vet. Parasitol. 311, 109788. https://doi.org/10.1016/j.vetpar.2022.109788.

Pincigher, L., Valenti, F., Bergamini, C., Prata, C., Fato, R., Amorati, R., Jin, X., Farruggia, G., Fiorentini, D., Calonghi, N., Zalambani, C., 2023. Myrcene: a natural compound showing anticancer activity in HeLa cells. Molecules 28, 6728. https://doi.org/10.3390/molecules28186728.

Reck, J., Klafke, G.M., Webster, A., DallAgnol, B., Schefer, R., Souza, U.A., Corassini, V. B., Vargas, R., Santos, J.S., Martins, J.R.S., 2014. First report of fluazuron resistance in *Rhipicephalus microplus*: a field tick population resistant to six classes of acaricides. Vet. Parasitol. 201, 128–136. https://doi.org/10.1016/j.vetpar.2014.01.012.

Ribeiro, P.H., Santos, M.L.D., Camara, C.A.D., Born, F.S., Fagg, C.W., 2015. Seasonal chemical compositions of the essential oils of two *Eugenia* species and their acaricidal properties. Quim. Nova 38, 3843. https://doi.org/10.5935/0100-4042.20150161.

Senra, T.O.S., Zeringóta, V., Oliveira Monteiro, C.M., Calmon, F., Maturano, R., Gomes, G.A., Faza, A., Carvalho, M.G., Daemon, E., 2013. Assessment of the acaricidal activity of carvacrol, (*e*)-cinnamaldehyde, trans-anethole, and linalool on larvae of *Rhipicephalus microplus* and *Dermacentor nitens* (Acari: Ixodidae). Parasitol. Res. 112, 1461–1466. https://doi.org/10.1007/s00436-013-3289-3.

Silva, J.K.R., Andrade, E.H.A., Barreto, L.H., Silva, N.C.F., Ribeiro, A.F., Montenegro, R. C., Maia, J.G.S., 2017. Chemical composition of four essential oils of *Eugenia* from the Brazilian Amazon and their cytotoxic and antioxidant activity. Medicines 4, 51. https://doi.org/10.3390/medicines4030051.

Silva Lima, A., Menezes, M., Freitas, M.G., Pinheiro, R., da Silva, R., Cavalcanti, M.V., Figueiredo, L., Borges, M., da Silva, E.J., Miranda, R., 2021. Acaricide activity of extract and an isolated compound of L*thraea brasiliensis* on *Rhipicephalus microplus* and selectivity actions against a non-target organism. Vet. Parasitol. 300, 109597. https://doi.org/10.1016/j.vetpar.2021.109597.

Silveira, R.M., Menezes, F.H., Lima, I.G., Carvalho, A.F.U., Oliveira Bünger, M., Costa, I. R., 2023. Essential oil constituents as the chemosystematic markers in E*ugenia* L. (Myrtaceae): An evolutionary perspective. S. Afr. J. Bot. 160, 309–318. https://doi.org/10.1016/j.sajb.2023.07.015.

Singh, N.K., Miller, R.J., Klafke, G.M., Goolsby, J.A., Thomas, D.B., Leon, A.A.P., 2018. In vitro efficacy of a botanical acaricide and its active ingredients against larvae of susceptible and acaricide-resistant strains of *Rhipicephalus (boophilus) microplus* canestrini (Acari: Ixodidae). Ticks Tick. Borne Dis. 9, 201–206. https://doi.org/10.1016/j.ttbdis.2017.09.005.

Tucker, A.O., Maciarello, M.J., Salywon, A.M., Landrum, L.R., 2001. Volatile leaf oil of *Calyptranthes sintenisii* kiaersk. (Myrtaceae) Of Puerto Rico. J. Essent. Oil Res. 13, 427–428. https://doi.org/10.1080/10412905.2001.9699715.

Van Den Dool, H., Kratz, P., 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. 11, 463–471.

Veras, B.O.D., Felix, R.F., Moura, G.M.D.M., Navarro, D.M.A.F., Aguiar, J.C.R.O.F., Wanderley, A.G., Silva, M.V., Correia, M.T.D.S., 2024. Essential oil of E*ugenia stictopetala* mart. Ex DC. Has antioxidant, antibacterial, and gastrointestinal protective effect. Nat. Prod. Res. 1–5. https://doi.org/10.1080/14786419.2024.2342556.

Webb, B., Sali, A., 2016. Comparative protein structure modeling using MODELLER, 6.37 Curr. Protoc. Bioinform 54 (5.6), 1–5. https://doi.org/10.1002/cpbi.3.

World Flora Online (WFO), 2024. *Eugenia stictopetala* DC. Published on the Internet. ⟨http://www.worldfloraonline.org/taxon/wfo-0000959144⟩ (accessed 7 Nov. 2024).

Wu, H.Q., Li, J., He, Z.D., Liu, Z.G., 2010. Acaricidal activities of traditional Chinese Medicine against the house dust mite, *Dermatophagoides farinae*. Parasitology 137, 975–983. https://doi.org/10.1017/S0031182009991879.

Wu, H.T., Li, D.M., Zhu, B.W., Du, Y., Chai, X.Q., Murata, Y., 2013. Characterization of acetylcholinesterase from the gut of sea cucumber *Stichopus japonicus*. Fish. Sci. 79 (2), 303–311. https://doi.org/10.1007/s12562-012-0588-z.

Yusuf, D., Davis, A.M., Kleywegt, G.J., Schmitt, S., 2008. An alternative method for the evaluation of docking performance: RSR vs RMSD. J. Chem. Inf. Model 48, 1411–1422. https://doi.org/10.1021/ci800084x.

Zhou, X., Xia, Y., 2009. Cloning of an acetylcholinesterase gene in *Locusta migratoria manilensis* related to organophosphate insecticide resistance. Pest. Biochem. Physiol. 93 (2), 77–84. https://doi.org/10.1016/j.pestbp.2008.11.007.