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Comparative Analysis of Essential Oil from the Leaves of Seven Species of the Genus *Eugenia* L. (Myrtaceae)

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Abstract

The genus *Eugenia* (Myrtaceae) is widely distributed in Brazil and is known for producing diverse secondary metabolites with various biological activities, although several species remain poorly explored. This study aimed to characterize the chemical composition of essential oils (EOs) from the leaves of seven *Eugenia* species (*E. brasiliensis*, *E. involucrata*, *E. longipedunculata*, *E. myrcianthes*, *E. neoverrucosa*, *E. pyriformis*, and *E. uniflora*), compare their chemical profiles using multivariate analysis, and evaluate their insecticidal activity against the flea *Ctenocephalides felis felis*. EOs were obtained from dried leaves by hydrodistillation using a Clevenger apparatus and analyzed by gas chromatography–mass spectrometry (GC–MS). Principal component analysis (PCA) was applied to compare chemical compositions, and contact bioassays were conducted to assess insecticidal activity against adult fleas. The EOs showed distinct chemical compositions, with major constituents including *α*-pinene, (*E*)-caryophyllene, viridiflorene, *β*-selinene, limonene, and germacrone, depending on the species. PCA revealed clear differences among species, particularly highlighting oils dominated by *α*-pinene and sesquiterpene-derived compounds. In the bioassays, *E. uniflora* showed the highest insecticidal activity, reaching 95.1% mortality at 800 μg·cm^{-2} and presenting an LC_{50} of 9.12 μg·cm^{-2}, whereas *E. brasiliensis* showed moderate activity (LC_{50} = 157.82 μg·cm^{-2}). These findings expand the chemical knowledge of the genus and indicate the potential of *E. uniflora* EO as a natural source of compounds with insecticidal activity against *C. felis felis*.

Keywords: GC-MS; Insecticidal; Myrtaceae; PCA

1. Introduction

Ctenocephalides felis felis is the most common flea species found in cats and dogs. These ectoparasites have a remarkable ability to jump and feed on their hosts' blood, from which they may remain for several consecutive days. In addition to causing skin allergies in pets, they can act as vectors of pathogens harmful to humans and other animals, including the Gram-negative bacteria *Rickettsia felis* and *Bartonella henselae*. These microorganisms are associated with typhus-like illness,

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flea-borne spotted fever, and cat-scratch disease in humans, respectively, representing a relevant public health concern [1–4].

Several classes of natural products contain compounds with antifeedant, insecticidal, and repellent properties, such as alkaloids, flavonoids, and terpenoids. Essential oils (EOs) are primarily composed of monoterpenes, sesquiterpenes, and phenylpropanoids [1,4]. Previous studies have reported the insecticidal potential of Eos from *Cannabis sativa* and *Piper aduncum* L. against *C. felis felis* [5,6].

The genus *Eugenia* L. (Myrtaceae) comprises approximately 1,239 accepted species worldwide [7] and is widely distributed in Brazil, with around 421 species [8]. Species of this genus are known to contain diverse chemical classes, including flavonoids, phenolic acids, tannins, and terpenoids [9]. Numerous *Eugenia* species have had their EOs characterized and described in the literature, and these findings will be discussed in further detail in the Discussion section [9,10].

Eugenia species have demonstrated biological activity against different types of parasites. *Eugenia uniflora* L. exhibits anti-*Leishmania* activity [11], while *Eugenia stipitata* McVaugh shows larvicidal and pupicidal effects against *Aedes aegypti*, with LC_{50} values of 0.34 mg/mL and 2.33 mg/mL, respectively [12]. The (EO) from the leaves and fruits of *Eugenia langsdorffii* O. Berg has also been reported to possess acaricidal activity against *Tetranychus urticae* [13]. Despite evidence of antiparasitic activity in some *Eugenia* species, no study has evaluated the genus against *C. felis felis.*

The aims of this study are: (i) to identify compounds not previously described for *Eugenia longipedunculata* Nied.; (ii) to analyze the chemical profiles of seven *Eugenia* species by gas chromatography coupled with mass spectrometry (GC–MS); (iii) to compare the profiles using principal component analysis (PCA); and (iv) to evaluate the insecticidal activity against *C. felis felis*, which has not yet been studied for this genus. Therefore, the chemical investigation of *Eugenia* species is relevant for generating new data on understudied taxa, for comparing their chemical profiles with regionally related species, and for contributing to the understanding of biological activities not yet reported in the literature for the parasite evaluated.

2. Results

2.1. Chemical Composition of Essential Oil

It is noted that of *Eugenia* species: *E. brasiliensis* Lam.; *E. longipedunculata*; *E. neoverrucosa* Sobral. and *E. uniflora* have higher yields (Table 1). *Eugenia neoverrucosa*, stands out, presenting a yield of 0.90%. Species with fewer oil-secreting cavities show low yields; this characteristic was observed in *E. involucrata* DC., *E. myrcianthes* Nied., and *E. pyriformis* Cambess.

Table 1. Essential oil yield observed in seven species of *Eugenia* genus.

	Mass (g)	Yield (mL)	Yield (%)
E. brasiliensis	806.35	2.4	**0.30**
E. involucrata	2577.69	2.3	**0.09**
E. longipedunculata	356.80	1.2	**0.34**
E. myrcianthes	1543.98	1.3	**0.08**
E. neoverrucosa	498.61	4.5	**0.90**
E. pyriformis	2370.23	2.3	**0.1**
E. uniflora	829.65	4.5	**0.55**

Source: The autor, 2025.

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According to Table 2, the major components identified are *α*-pinene (20.51%), (*E*)-caryophyllene (17.52%) and 1,8 cineole (17.01%) in *E. brasiliensis*; (*E*)-caryophyllene (25.59%), viridiflorene (26.32%) and aromadendrene (18.96%) in *E. involucrata*; (*E*)-caryophyllene (19.19%) and viridiflorene (9.31%) in *E. longipedunculata*; *β*-selinene (22.88%), *α*-guaiene (16.23%), *δ*-amorphene (12.21%) and *β*-elemene (9.73%) in *E. myrcianthes*; *α*-pinene (79.92%) in *E. neoverrucosa*; *α*-pinene (32.94%) and limonene (24.56%) in *E. pyriformis*, and the germacrene-type sesquiterpenoids germacrone (26,48%), atractylone (11.08%) and curzerene (9.94%) in *E. uniflora*, Figure 1. Both hydrocarbon and oxygenated mono- and sesquiterpenes were identified. Minor compounds are listed in Table 2.

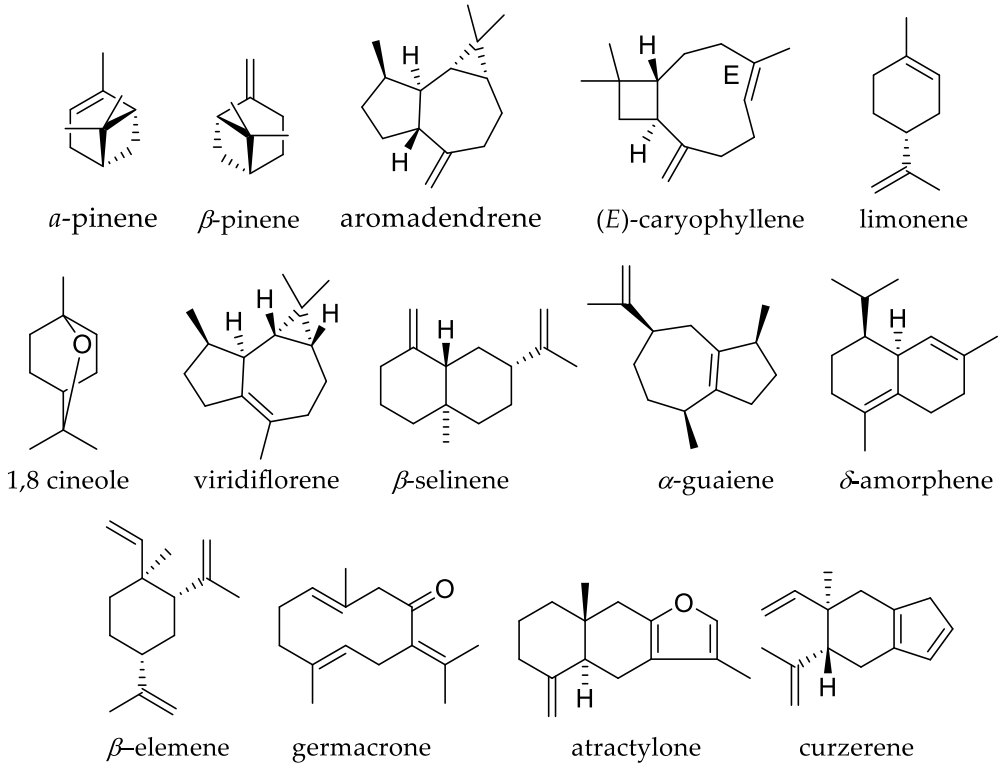

Figure 1. Major structures in *Eugenia* species.

Table 2. Chemical composition of essential oils from the leaves of *Eugenia* species.

Compounds	IRL	*E. brasiliensis*	*E. involucrata*	*E. longipedunculata*	*E. myrcianthes*	*E. neoverrucosa*	*E. pyriformis*	*E. uniflora*
Hexanol <2->	796	2.18	2.07	-	1.3	-	3.2	-
Hexenal <(2*E*)->	846	0.73	-	-	0.12	-	-	-
Hexenol <(3*Z*)->	850	0.67	-	-	-	-	-	0.44
Santene	884	-	-	-	-	-	2.1	-
α-Pinene	932	**20.51**	-	**7.76**	-	**81.9**	**32.94**	-
Camphene	946	-	-	-	-	0.32	-	-
β-Pinene	974	-	-	-	-	3.71	2.78	-
Myrcene	988	-	-	-	1.03	0.37	2	-
δ-Carene	1001	0.96	-	-	-	-	0.58	-

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α-Phellandrene	1002	-	-	-	-	-	2.8	-
ρ-Cymene	1020	5.9	-	-	-	-	-	-
Limonene	1024	3.14	-	1.42	-	2.67	**24.56**	-
1,8-Cineole	1026	**17.01**	-	5.99	-	-	0.41	-
β-Ocimene	1032	-	-	-	-	-	3.36	-
α-Campholenal	1122	-	-	-	-	-	**6.44**	-
γ-Terpinene	1054	2.08	-	1.01	-	0.27	-	-
Isoborneol	1155	-	-	-	-	-	1.87	-
γ-Terpineol	1162	-	-	-	-	3.9	-	-
Borneol	1165	0.72	-	-	-	-	-	-
α-Terpineol	1186	1.25	-	-	-	0.27	-	-
Cubebene	1348	2.38	0.64	-	2.27	0.53	0.6	3.35
Isoledene	1374	-	0.28	-	-	-	-	1.26
α-Copaene	1376	-	-	3.32	-	-	-	-
β-Panasinsene	1381	-	0.96	-	-	-	-	-
β-Elemene	1389	-	3.39	1.55	**9.73**	-	-	1.29
Bornyl acetate	1284	-	-	-	-	0.25	-	-
α-Ylangene	1373	-	-	-	-	1.52	-	-
(*E*)-Caryophyllene	1417	**17.52**	**25.59**	**19.19**	**6.97**	4.29	**8.35**	5.28
α-Guaiene	1437	-	-	-	**16.23**	-	4.02	-

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Aromadendrene	1439	-	**18.96**	3.93	-	-	-	-
(*Z*)-Farnesene	1440	0.66	-	-	-	-	-	-
cis-Muurola-3.5-diene	1448	1.99	0.66	-	-	-	-	-
Himachalene	1449	-	-	-	-	-	-	0.09
trans-Muurola-3.5-diene	1451	-	-	-	-	-	1	-
α-Humulene	1452	2.04	3.03	2.75	3.16	-	-	-
β-Farnesene	1454	-	-	-	-	-	0.1	-
Aromadendrene <allo->	1458	-	-	-	0.56	-	2.9	4.58
Aromadendrane <dehydro->	1460	-	-	4.37	-	-	-	-
Caryophyllene <9-epi-(*E*)->	1464	-	-	1.8	-	-	-	-
γ-Gurjunene	1475	-	-	-	3.1	-	-	-
γ-Muurolene	1478	-	-	-	5.7	-	-	0.39
Germacrene D	1480	0.78	1.16	-	-	-	-	2.76
α-Amorphene	1483	-	-	-	0.22	-	-	0.4
β-Selinene	1489	-	**7.36**	0.74	**22.88**	-	-	1.01
d-Selinene	1492	-	-	0.65	-	-	-	-
γ-Amorphene	1495	1.42	-	2.49	1.3	-	-	0.59
Viridiflorene	1496	2.01	**26.32**	**9.31**	-	-	-	-
Curzerene	1499	-	-	-	-	-	-	**11.2**
α-Muurolene	1500	-	-	0.89	0.71	-	-	1.45

 doi:10.20944/preprints202603.1780.v1

Guaiene <trans-β->	1502	-	-	1.72	-	-	-	-
β-Bisabolene	1505	5.81	0.57	6.83	-	-	-	-
Germacrene A	1508	-	-	-	0.85	-	-	-
δ-Amorphene	1511	-	-	4.4	**12.21**	-	-	**7.51**
Selinene <7-epi-α->	1520	-	-	-	0.17	-	-	-
δ-Cadinene	1522	2.66	3.96	-	-	-	-	-
Zonarene	1529	-	-	0.74	-	-	-	-
Nerolidol	1531	-	-	-	-	-	-	1.57
α-Cadinene	1537	-	-	-	-	-	-	**6.36**
Selina-3.7(11)-diene	1545	-	-	-	-	-	-	2.98
Spathulenol	1577	4.87	-	1.6	-	-	-	-
Globulol	1590	-	-	3.57	-	-	-	-
Viridiflorol	1592	-	-	2.04	-	-	-	-
Cubeban-11-ol	1595	-	-	0.88	-	-	-	-
Rosifoliol	1600	-	-	1.82	-	-	-	-
1-epi-Cubenol	1627	-	-	1.81	-	-	-	-
Cedranone	1628	-	0.68	-	-	-	-	-
epi-α-Muurolol	1640	-	-	1.73	-	-	-	-
α-Cadinol	1652	-	-	-	1.37	-	-	-
Intermedol <neo->	1658	-	-	2.65	0.41	-	-	-
Atractylone	1657	-	-	-	-	-	-	**16.08**

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Selin-11-en-4-α-ol	1658	-	-	-	4.26	-	-	
Germacrone	1693	-	-	-	-	-	-	**26.48**
Hydrocarbons		3.58	2.07	0	1.42	0	3.2	0.44
Monoterpene		30.51	0	1.42	1.03	88.97	67.76	0
Monoterpene oxygenated		21.06	0	7.0	0	4.44	12.08	0
Sesquiterpene		2.38	5.27	4.87	12.0	0.53	0.6	5.9
Sesquiterpene oxygenated		39.76	88.29	75.91	80.1	6.06	15.37	88.73
Total		97.3	95.6	97.0	94.6	100	100	95.1

2.2. Comparative Analysis of Essential Oils from Eugenia Species Using Molecular Networking and Principal Component Analysis (PCA)

Correlation and multivariate analyses were conducted to highlight differences in chemical composition among the seven *Eugenia* species (Table 2) and the classes of compounds presented (Figure 2). Similarities among the EOs are evident in the PCA analysis (Figure 2). *E. involucrata* shows a stronger association with viridiflorene and (*E*)-caryophyllene, while *E. longipedunculata* appears closer to the central region, suggesting the presence of similar compounds but at different concentrations. *E. myrcianthes* and *E. uniflora* that shows the compounds, aromadendrene, γ-amorphene and α- murolene in common are located on the left side of the PCA plot, although they are separated along the second component, indicating differences in their chemical profiles. According to the graphic, *E. brasiliensis*, *E. neoverrucosa*, and *E. pyriformis* contain α-pinene and limonene, indicating that *E. neoverrucosa* has the highest α-pinene concentration, as already observed in Table 2.

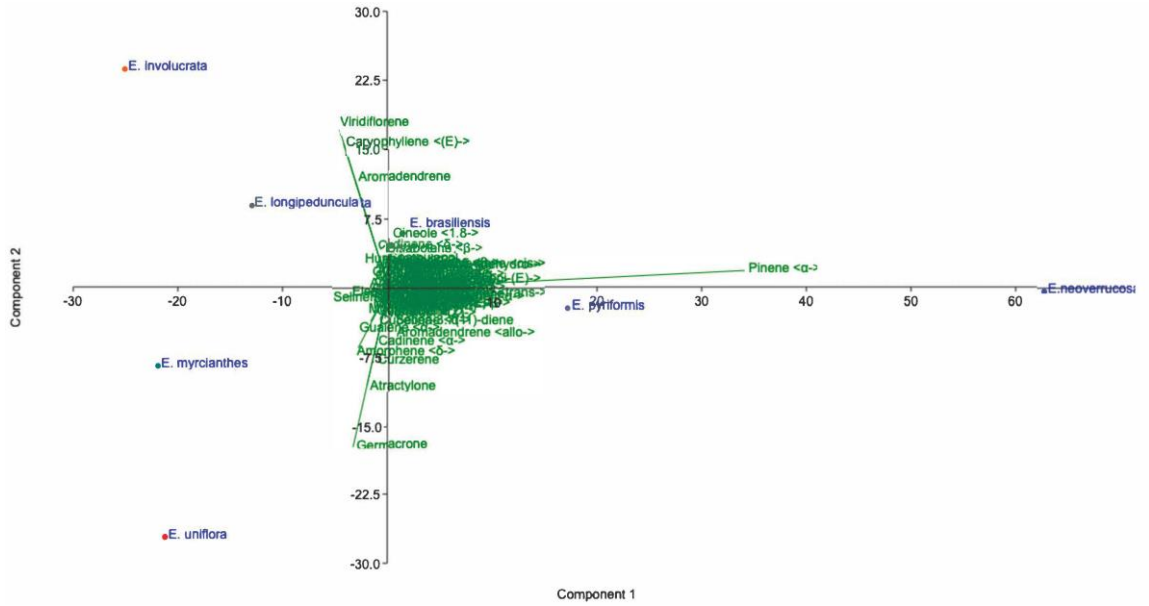

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Figure 2. Principal component analysis (PCA1 = 61.31, PCA2 = 15.17%, and PCA3 = 10.23) of 7 essential oil samples from *Eugenia* species, obtained by hydrodistillation and analyzed by GC–MS, and the correlation of all chemical components was identified in the analysis.

In Figure 3, the presence of hydrocarbons and monoterpene oxygenated is seen in *E. brasiliensis* and *E. pyriformis*, the latter being more prominent in *E. brasiliensis*. The species *E. involucrata*, *E. myrcianthes*, and *E. uniflora* indicate the presence of hydrocarbons, monoterpenes, and sesquiterpenes that are oxygenated in a very similar manner. *Eugenia neoverrucosa* shows the highest concentration of monoterpenes.

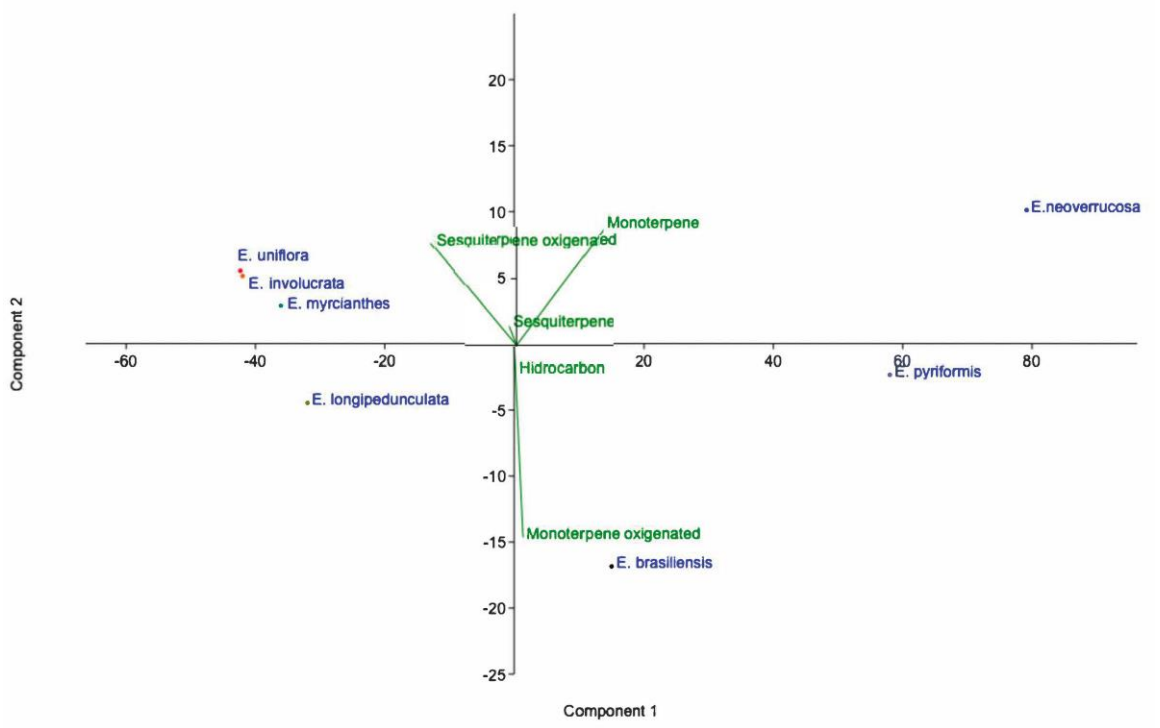

Figure 3. Principal component analysis (PCA1 = 80.21, PCA2 = 11.17%) of 7 essential oil samples from *Eugenia* species, obtained by hydrodistillation and analyzed by GC–MS, and the correlation of the presented class (Hydrocarbons, monoterpenes, monoterpenes oxygenated, sesquiterpene, and sesquiterpenes oxygenated).

2.3. Insecticidal Activity Against Adult Fleas

A general increase in insecticidal activity was observed with increasing concentration for most of the evaluated EOs, although the intensity and consistency of this response varied among species. The EO of *E. uniflora* was the most active across nearly the entire concentration range, achieving 95.1% mortality at 800 $\mu g.cm^{-2}$, demonstrating a strong dose–response relationship. The EO of *E. brasiliensis* showed moderate activity at low and intermediate concentrations but exhibited a marked increase at higher doses, achieving 80.0% mortality at 800 $\mu g.cm^{-2}$

In contrast, EOs *pyriformis*, *E. neoverrucosa*, and *E. myrcianthes* showed intermediate insecticidal activity, with mortalities of 60.0%, 52.4%, and 50.0%, respectively, at the highest tested concentration. Finally, *E. involucrata* was the least active oil among those evaluated, with a maximum mortality of 38.9% at 800 $\mu g.cm^{-2}$ (Table 3).

 doi:10.20944/preprints202603.1780.v1

Table 3. Insecticidal activity (%) of essential oils from *Eugenia* spp. against adults of *Ctenocephalides felis felis.*

Concentration (µg.cm⁻²)	*E. brasiliensis*	*E. involucrata*	*E. longipedunculata*	*E. myrcianthes*	*E. neoverrucosa*	*E. pyriformis*	*E. uniflora*
1.5	0.0	0.0	0.0	0.0	0.0	20.0	5.3
3	0.0	0.0	0.0	4.8	9.5	55.0	20.0
6	15.0	5.3	5.6	9.5	17.6	9.5	21.1
12	17.0	0.0	10.0	4.8	19.4	16.7	47.3
25	19.2	5.3	10.6	15.0	20.0	13.6	55.0
50	23.8	5.0	42.2	28.6	23.8	10.0	70.6
100	25.1	19.0	38.9	42.1	24.3	45.0	88.3
200	55.0	31.6	65.3	63.2	22.2	31.6	89.5
400	75.0	31.6	60.0	35.0	25.5	42.9	94.7
800	80.0	38.9	65.3	50.0	52.4	60.0	95.1

Note: *The positive control resulted in 100% mortality in all bioassays, while the negative control produced no mortality.*

Based on the mortality data, LC_{50} estimation was possible only for *E. uniflora* and *E. brasiliensis*. *E. uniflora* showed an LC_{50} of 9.12 µg.cm^{-2} (95% CI: 6.59–12.17), whereas *E. brasiliensis* presented an LC_{50} of 157.82 µg/cm² (95% CI: 116.16–225.18). The slope coefficients were 1.19 for *E. uniflora* and 1.09 for *E. brasiliensis*. Chi-square tests indicated no significant deviation from the model ($p \geq 0.999$), (Table 4).

Table 4. Lethal concentration (LC_{50}) values of essential oils from *Eugenia* uniflora and ***Eugenia brasiliensis*** obtained by probit analysis.

Essential Oil	LC_{50} (µg/cm²)	95% CI (Lower–Upper)	Slope ± SE	R^2	χ^2	*p-value*
Eugenia uniflora	**9.12**	**6.59–12.17**	**1.19 ± 3.86**	**0.911**	**13.240**	**1.000**
Eugenia brasiliensis	**157.82**	**116.16–225.18**	**1.09 ± 2.59**	**0.890**	**21.770**	**0.999**

3. Discussion

3.1. Chemistry of Essential Oils

According to the review by [14], the EO yield obtained from dried leaves of *E. brasiliensis* by hydrodistillation was 0.39%, which is similar to the value observed in this study and higher than yields obtained from fresh leaves (0.08–0.14%) [15]. For *E. involucrata*, yields of 0.45% and 0.21% from dried leaves have been reported ([16], which are higher than those obtained in the present study. [17] reported yields of 0.06% for *E. myrcianthes*, 0.42% for *E. neoverrucosa*, and 0.17% for *E. pyriformis*. In *E.*

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uniflora, yields reported in the literature vary widely (0.15–3.1%), and the yield obtained in this study (0.55%) falls within this range [14]. Overall, the yields obtained here are consistent with the literature, except for *E. neoverrucosa*, which exhibited a considerably higher yield.

Similar chemical profiles are observed in *E. brasiliensis*, *E. neoverrucosa* and *E. pyriformis*, particularly due to the presence of *α*-pinene, which was most abundant in *E. neoverrucosa.* The substance (*E*)-caryophyllene was common to *E. brasiliensis* and *E. longipedunculata.* Viridiflorene was found in *E. involucrata* and *E. longipedunculata*. Differences among species and variations in minor constituents are shown in Table 2.

The major compounds observed in *E. brasiliensis* have previously been reported by [18], whose samples collected from different cities revealed that this species generally presents the same major constituents. These authors identified *α*-selinene (13.3–14.8%) and *β*-selinene (12.6–17.3%) as the main compounds and reported the bicyclic sesquiterpene *(E)*-caryophyllene (8.7–12.6%), which was also reported in the present study. The remaining compounds were also detected, but only in minor amounts. In fresh leaves of *E. brasiliensis*, the major constituent was *α*-muurolol (12.1%), and *α*-pinene was not detected [19]. In contrast, *α*-pinene (15.94%) was identified as the major compound in winter leaves of *E. brasiliensis* [15]. Germacrene B (22.17±1.72), byciclogermacrene (19.76±1.28) and *β*-elemene (10.86±0.93) were detected as major compounds in *E. involucrata* by D'Almeida et al. (2021), elixene (26.53%) and caryophyllene (13.16%) were identified by [20]. This last compound corroborates with the current study: aromadendrene was found in minor amounts, and viridiflorene was not found in the studies mentioned.

There are no reports on the EO composition of *E. longipedunculata*; however, *(E)*-caryophyllene was common to other species examined in this study as well as to other species within the genus. The compound viridiflorene has been reported in dried leaves of *E. uniflora* [21] and *E. myrcianthes* [17,22] also reported *E. myrcianthes* (syn.: *Hexachlamys edulis* (O. Berg) Kausel & D. Legrand), in which the hydrocarbon sesquiterpene *β*-selinene (16.1%) was the major compound; *β*-elemene (1.0%) was detected in very small amounts, while the remaining constituents were not reported. The compound β-copaen-4-*α*-ol (31.7%) was identified by [17].

Nonpolar fractions of *E. myrcianthes* showed the following volatile compounds: in the hexane fraction, the triterpenes lupenyl acetate (45.39%), *β*-amyrone (12.69%), and squalene (12.18%); the cadinane sesquiterpenoid *τ*-muurolol (10.80%); viridiflorene (7.21%); and the tricyclic sesquiterpenoid spathulenol (4.57%). In the chloroform fraction, *δ*-cadinene (9.0%) and *α*-muurolene (4.15%) were found, which were found in this study with 0.71% [23]. Different compounds were observed in extractions using non-polar compounds, with sesquiterpenes and triterpenes predominating, whereas a distinct profile was obtained from Clevenger hydrodistillation in the present work.

In *E. neoverrucosa*, [17] reported a higher concentration of *α*-pinene at 94.5%. *E. pyriformis* was found to contain the bicyclic monoterpene isomers *β*-pinene (39.7%) and *α*-pinene (31.5%) as major compounds in previous research [17]. Hydrodistillation of dried leaves identified *β*-caryophyllene (17.82%), bicyclogermacrene (12.84%), and globulol (5.96%) [24]. A seasonal analysis showed *β*-pinene as the most prevalent, with levels fluctuating between 0.2% and 25.7%, peaking in January. *α*-Pinene was present in small amounts (0.5-7.4%), and limonene varied from 0.3% to 22.0%, peaking in October [25].

This study identified high levels of *α*-pinene and limonene, along with a small amount of *β*-pinene (2.78%). While seasonality was not examined here, the same compounds were present. *E. uniflora* EO composition has been widely studied using both fresh and dried leaves. The main compounds identified by hydrodistillation of dried leaves generally align with those reported in this study, though their proportions differ across studies. Frequently reported compounds include germacrone, curzerene, germacrene B, caryophyllene oxide, spathulenol, and *α*-selinene [14, 21, 26). Additionally, atractylone, a sesquiterpenoid identified here, has been detected in minor amounts in other samples [14,26,27].

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3.2. Insecticidal Activity of the Essential Oils

Among the species tested, only *E. brasiliensis* and *E. uniflora* demonstrated insecticidal activity against adult *C. felis felis*. *E. uniflora* achieved higher mortality rates than *E. brasiliensis* at concentrations of 5,000; 10,000; 20,000; and 40,000 µg/mL, with mortality rates of 88.3%, 89.5%, 94.7%, and 95.1%, respectively. Meanwhile, *E. brasiliensis* resulted in mortalities of 25.1%, 55.0%, 75.0%, and 80.0% at the same concentrations. Correspondingly, the LC_{50} for *E. uniflora* was about 17 times lower than that of *E. brasiliensis* (see Table 4), indicating much higher toxicity. These results suggest that *E. uniflora* has greater insecticidal potential than *E. brasiliensis* against adult *C. felis felis*.

Recent studies have highlighted the potential of plant-derived essential oils for control parasite infestations in dogs and cats, including *C. felis felis* (fleas). EOs from *Alpinia zerumbet* B.L. Burtt & R.M. Smith, *Cinnamomum* spp., *Cymbopogon nardus* (L.) Rendle, *Laurus nobilis* L., *Mentha spicata* L., and *Ocimum gratissimum* L. have demonstrated activity against *C. felis felis* at different developmental stages, including adults, larvae, and eggs [28]. In the species reported 1,8 cineole (eucalyptol) was the major compound found in *A. zerumbet*, *O. gratissimum* and *L. nobilis*, which was seen in *E. brasiliensis*. 1,8-cineole (24.11%), camphor (12.13%), and curzerenone (9.68%) were detected in the EO of the fresh rhizomes of *Curcuma zedoaria* (Christm.) Roscoe and presented 100%, 94.23% mortality, 100% and 98% of mortality against adult fleas, pupal stages, larvae and eggs of *C. felis felis*, respectively at the concentrations of 800 µg.cm^{-2}, 396 µg.cm^{-2}, 117,5 µg.cm^{-2} and 396 µg.cm^{-2} [29].

The EO from fresh leaves of *Piper aduncum* L., rich in the phenylpropanoid dillapiole (77.56–85.52%), promoted 100% mortality of flea eggs at 100 µg/mL and adults at 1,000 µg/mL [6], a chemical profile that was not observed in the present study. [30] demonstrated that EO from *Baccharis trimera* (Less.) DC. and *Mimosa verrucosa* Benth exhibited strong insecticidal activity against adult fleas, achieving 100% mortality at 800 µg·cm^{-2}, with residual activity lasting up to three days and low toxicity (LC_{50} = 369.22 µg·cm^{-2}). Interestingly, *M. verrucosa* contains *α*-pinene and (*E*)-caryophyllene as major constituents, compounds also identified in *E. brasiliensis*. The major compounds found in *E. brasiliensis* and *E. uniflora* have previously been associated with antiparasitic activity in other organisms. The compound *(E)*-caryophyllene isolated from *Ageratum conyzoides* L. and tested orally in cross-bred male calves was effective against tick species, such as the IVRI-I strain of *Rhipicephalus microplus, R. annulatus* and *Hyalomma anatolicum* [31].

The EO of *Psidium brownianum* Mart. ex DC. contains isogermafurene (52.93%) and germacrone (16.02%), and the tested oil inhibited the parasites *Leishmania braziliensis* (92.61%), *Leishmania infantum* (84.16%) and *Trypanosoma cruzi* (57.05%) in 1,000 µ/mL [32]. Additionally, Pretel et al. (2019) synthesized different germacrene-derived compounds, which the compound 7,11-epoxieudesma-3,7(11)-dien-8-one exhibited insecticidal activity against the aphid *Rhopalosiphum padi*, a plant parasite, while, 1,10-epoxygermacrone showed acaricidal activity against the tick *Hyalomma lusitanicum,* and demonstrated greater activity than germacrone. These findings suggest that germacrone, identified in *E. uniflora* in the present study, may also serve as a promising precursor for bioactive derivatives.

The chemical component *α*-pinene *α*-Pinene has been reported to cause toxicity by fumigation and contact exposure in *Sitophilus zeamais*, with an LC_{50} value of 4.133 ppm after 14 days [33]. This compound has also shown activity against the cattle tick *Rhipicephalus microplus* at high concentrations [34], supporting its potential contribution to the insecticidal activity observed in the present study.

Despite the promising results, the use of essential oils in animals requires caution, as adverse reactions such as depression, incoordination, muscle tremors, pruritus, scratching, and weakness have been reported in pets [35,36]. Therefore, the findings presented here should be considered preliminary, highlighting the need for further toxicological and formulation studies before practical applications involving *Eugenia* essential oils.

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4. Materials and Methods

Species of the genus *Eugenia* were collected in the state of Paraná, southern Brazil, within the Atlantic Forest biome. Botanical identification was performed, and voucher specimens were deposited in the Herbarium of the State University of Ponta Grossa, as detailed in Appendix A, Table A1. The collections were registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration code ADDD35A. Plant material for essential oil extraction was collected in March 2025.

4.1. Preparation of Plant Material and Essential Oil Extraction

For the procedures below, the leaves were dried in an oven (LUCA-82, Lucadema ®) at 26 ± 4 ◦C and crushed in a blender. They were immediately subjected to essential oil extraction. The EO extraction was performed in a Clevenger apparatus according to the [37], at a gentle boil of 40 ºC for two hours.

4.2. Gas Chromatography Coupled with Mass Spectrometry (GC-MS) Analyses

Chemical composition was determined using a Gas Chromatograph 5890 Series II (Agilent, USA), equipped with a flame ionization detector and an injector in the "split" mode (1:20). The substances were separated on a fused silica capillary column DB-5 (30 m x 0.25 mm x 0.25 μm). Helium (He) was used as the carrier gas with a flow rate of 1.0 mL. min^{-1}. The temperature program used in the oven was 60 ◦C for 2 min with an increment of 5 ◦C min^{-1} up to 110 ◦C, followed by an increment of 3 ◦C.min^{-1} up to 150 ◦C, and finally, an increment of 15 ◦C.min^{-1} up to 290 ◦C, held for 15 min. The injector and detector temperatures were set at 220 ◦C and 290 ◦C, respectively. A Gas Chromatograph coupled to a Mass Spectrometer QP-2010 Plus (Shimadzu, JPN) was used to separate and analyze the substances present in EO. The helium flow rate, capillary column, and temperature program for GC-MS analysis were the same as described for GC-FID analysis. The injector and interface temperatures were set at 220 ◦C and 250 ◦C, respectively. Mass spectra were obtained with a quadrupole detector operating at 70 eV, over a mass range of 40-400 m/z, at a scan rate of 0.5 $scans^{-1}$. The components were identified by calculating retention linear indices (IRLs) that correlated the retention times of the compounds with those of a homologous series of n-alkanes (C7-C30). The mass spectral fragmentation patterns were compared with data from the literature [38] and the equipment database (NIST Library, 2008).

4.3. Insecticidal Activity Against Ctenocephalides Felis Felis

For each replicate, ten unfed adult fleas (five males and five females), 14 days post-emergence from the pupal stage, of the subspecies *Ctenocephalides felis felis* were used. Fleas originated from a laboratory colony maintained on cats, with approval from the Animal Use Ethics Committee (CEUA-IV-UFRRJ), protocol number 4313110419.

EOs of *Eugenia* spp. were diluted in analytical-grade acetone and prepared through a 1:2 serial dilution to obtain a range of ten concentrations from 40,000 to 78.125 μg/mL. For the bioassay, filter paper strips (Whatman No. 1, 80 g; area = 10 cm^2) were impregnated with 200 μL of each solution. After impregnation, papers were left on the bench for at least 30 min to allow solvent evaporation. Final concentrations corresponded to the mass of EO remaining on the filter paper after acetone evaporation, resulting in a surface concentration range of 800 to 1.5 μg/cm^2.

Each concentration was tested in six replicates. A negative control consisting of filter paper strips treated with acetone only was included to confirm the absence of vehicle effects. A positive control was performed using fipronil at 8 μg/cm^2.

Bioassays were conducted to evaluate insecticidal activity by contact exposure of adult fleas. After drying, impregnated filter paper strips were placed inside test tubes, and fleas were introduced. Tubes were maintained in a climatic chamber at 27 ± 1 °C and 70 ± 10% relative humidity for 24 h.

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After the exposure period, specimens were examined under a stereomicroscope to determine the biological effect. Mortality was assessed based on motility; individuals showing no movement were considered dead. Mortality percentage was calculated for each concentration according to the formula:

Mortality (%) = 100 × (number of dead individuals / total individuals exposed)

Lethal concentrations causing 50% (LC_{50}) and 90% (LC_{90}) mortality were estimated by probit analysis. Statistical analyses were performed in RStudio (R Core Team) using the ecotoxicoly package, with a 95% confidence interval.

4.4. Data Analysis

Principal component analysis and hierarchical grouping were performed using the PAST program, version 3.13.12. The data used for the multivariate analyses were the dependent variables compounds of the EOs and classes such as monoterpene hydrocarbons (MH), oxygenated monoterpenes (OM), sesquiterpene hydrocarbons (SH), oxygenated sesquiterpenes (OS), and diterpene (DIT), while the independent variables were essential oil samples based on species.

5. Conclusions

The findings of this work demonstrate significant chemical variability among EOs obtained from the leaves of the *Eugenia* species studied, with monoterpenes and oxygenated sesquiterpenes as the predominant constituents. Major compounds such as α-pinene, (*E*)-caryophyllene, viridiflorene, β-selinene, aromadendrene, limonene, and germacrone characterized distinct chemical profiles among species, as confirmed by principal component analysis. Among the evaluated samples, *E. neoverrucosa* presents the highest essential oil yield, while *E. uniflora* exhibited a distinctive composition rich in germacrone-type sesquiterpenoids. In the insecticidal assays against adult *C. felis felis*, *E. uniflora* shows the highest activity, reaching 95.1% mortality at 800 $\mu g \cdot cm^{-2}$ and presenting the lowest LC_{50} (9.12 $\mu g \cdot cm^{-2}$), whereas *E. brasiliensis* displays moderate activity, *E. pyriformis*, *E. neoverrucosa*, and *E. myrcianthes* show intermediate activity and *E. involucrata* demonstrate lower effects. These findings expand the chemical knowledge of the genus and represent the first report of the EO composition of *E. longipedunculata*, and the insecticidal activity of *Eugenia* EOs against *C. felis felis*, highlighting *E. uniflora* as a promising source of natural compounds for ectoparasite control, although further studies on toxicity, active constituents, and formulation are still required.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1-S7.

Author Contributions: Conceptualization, methodology, formal analysis, investigation, writing—original draft preparation, writing—review and editing, L.A.; formal analysis, investigation, N.F.P.; formal analysis, investigation, writing—original draft preparation, D.R.C.; formal analysis, investigation, Y.P.C.; methodology, writing—original draft preparation, I.T.M.; formal analysis, investigation, N.M.M.E.; data curation, investigation, writing—original draft preparation, writing—review and editing, D.S.A.C.; supervision, project administration, writing—review and editing, J.M.

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Abbreviations

The following abbreviations are used in this manuscript:

EOs	Essential oils
GC-MS	gas chromatography–mass spectrometry
PCA	Principal component analysis

Appendix A

Table A1. Data of collection of the *Eugenia* species.

Species	Popular name	Site of collection	Coordinates	Register
E. brasiliensis	grumixama, grumixameira ibaropoti, cumbixaba	Ponta Grossa	25°07′30.2″S 50°09′25.5″W	HUPG23392
E. involucrata	cereja-do-mato, cerejeira-do-mato, cereja-do-rio-grande	Ponta Grossa	25°05′42.0″S 50°09′42.8″W	HUPG 2824
E. longipedunculata	pitanga-laranja, grumixama-mirim, grumixama-da-mata, grumixama-miúda	Ponta Grossa	25°07′30.2″S 50°09′25.5″W	HUEPG23518
E. myrcianthes	pessegueiro-do-mato, ubajaí,	Carambeí	24°54′35.9″S 50°09′41.5″W	HUEPG23349
E. neoverrucosa	ibirubá, guamirim-ripa, ibacurú, araçá-ripa	Ponta Grossa	25°15′10.8″S 50°00′06.1″W	HUEPG23391
E. pyriformis	uvaia, uvalha, uvaieira, uvalheira	Carambeí	25°07′30.2″S 50°09′25.5″W	HUEPG23346

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E. uniflora	pitanga, pitangueira	Ponta Grossa	25°07'30.2"S 50°09'25.5"W	HUEPG23347

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