

Apiol and Dillapiole Dominate the Essential Oil Profile of a Single Collection of *Piper macedoi* from the Brazilian Atlantic Forest and Confer Cytotoxic and Antibacterial Activities

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Research Article

Keywords: Piper macedoi, apiol, dillapiol, chemical variability, cytotoxicity, antibacterial activity, in silico prediction

Posted Date: June 2nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9361751/v1>

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Additional Declarations: No competing interests reported.

Abstract

The essential oil extracted from the leaves of *Piper macedoi* Yunck., collected in the Atlantic Forest of southern Bahia (Brazil), was analyzed via gas chromatography coupled with mass spectrometry (GC–MS), revealing a chemical profile dominated by the arylpropanoid apiole (39.81%) and dillapiole (26.47%). This profile represents an atypical occurrence for the species, which is distinct from most previously reported profiles. Notably, this compositional pattern was not observed in subsequent collections, suggesting nonstable, context-dependent expression. The oil exhibited high cytotoxicity against *Artemia salina* nauplii (LD₅₀ = 123.33 µg/mL; 95% CI: 93.34–157.15 µg/mL) and selective antibacterial activity against *Escherichia coli*, with inhibition halos of 5.33–10.33 mm across all tested concentrations, whereas the inhibition of *Staphylococcus aureus* was restricted to the highest concentrations. *In silico* analyses via the Prediction of Activity Spectra for Substances (PASS) platform and GUSAR software indicated that apiole and dillapiole are apoptosis agonists (Pa > 0.73) and caspase-3 stimulants (Pa > 0.85) and predominantly possess gram-negative antibacterial targets. The predicted acute oral toxicity was low (OECD Class 5; LD₅₀ > 2000 mg/kg), whereas the intravenous route showed considerably greater toxicity (OECD Class 3; LD₅₀ ≈ 39 mg/kg). These results suggest that this atypical chemical profile of *P. macedoi* is associated with broad bioactive potential, warranting further investigation of its pharmacological and ecological properties.

1. Introduction

Essential oils from plants are complex mixtures of volatile secondary metabolites with a broad spectrum of biological activities, including antimicrobial, antifungal, insecticidal, and cytotoxic properties [1]. A fundamental aspect of these natural products is the intraspecific variability in their chemical composition: different populations of the same species may accumulate distinct sets of major compounds, reflecting the metabolic plasticity of plants in response to evolutionary and environmental pressures [2]. This variability has direct implications for evaluating the biotechnological potential of plant species, as it necessitates the chemical characterization of samples from different localities, seasons, and growth conditions.

The essential oils of plants belonging to the genus *Piper* L. (Piperaceae) are characterized by the presence of arylpropanoids such as apiole, dillapiole, safrole, myristicin, and elemicin, in addition to mono- and sesquiterpenes in variable proportions. These metabolites perform relevant ecological functions within the genus, including defense against herbivores, attraction of pollinators, and modulation of microbial interactions, indicating the unique value of *Piper* as a model for studies of chemical ecology and the biogeography of secondary metabolites [3].

Piper macedoi Yunck. is a species endemic to the Brazilian Atlantic Forest. Available studies on its essential oil reveal considerable intraspecific chemical variability: whereas the majority of reports describe profiles dominated by monoterpenes such as β-pinene, 1,8-cineole, and α-pinene or by sesquiterpenes such as bicyclogermacrene and β-caryophyllene [4, 5], some samples exhibit dominance of arylpropanoids, notably apiole and dillapiole [6, 7]. The apiole/dillapiole-rich profile, when present, represents a substantial fraction of the total composition and a distinct compositional pattern with specific bioactive potential. The rigorous description and contextualization of an atypical chemical profile observed in a specific collection, including its environmental context and biological implications, constitute a relevant scientific gap that this study aims to address.

The general objective of this study was to investigate an atypical chemical profile rich in apiole and dillapiole, observed in a specific collection of the essential oils of *P. macedoi*, from chemical, ecological, and pharmacological perspectives. The specific objectives were as follows: (i) characterize the chemical composition of the essential oil via gas chromatography–mass spectrometry (GC–MS)/gas chromatography (GC–FID) coupled with flame ionization detection and contextualize the obtained profile in relation to the intraspecific variability reported in the literature; (ii) evaluate the cytotoxicity of the oil against *Artemia salina* nauplii; (iii) determine the antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* via the disc diffusion method; and (iv) perform *in silico* predictions of biological activities, acute toxicity, and molecular targets for the major compounds apiole and dillapiole via the PASS and GUSAR platforms.

2. Materials and methods

2.1. Plant material collection and identification

The collection of Piperaceae species was authorized by the System of Authorization and Information on Biodiversity (SISBIO, license no. 31544). Fresh leaves of *P. macedoi* Yunck. were collected from a remnant of the Atlantic Forest at Fazenda Palmeira, municipality of Teixeira de Freitas, Bahia, Brazil (17°25′29.4″S, 39°41′11.6″W). The voucher specimen was taxonomically identified by Dr. Elsie F. Guimarães (Instituto de Pesquisa Jardim Botânico do Rio de Janeiro) and deposited at the Jardim Botânico herbarium under accession number RB 73273.

2.2. Environmental Context of the Collection Site

The collection was carried out in January 2017, corresponding to the austral summer (rainy season) in the Atlantic Forest biome of northeastern Brazil. To characterize the environmental conditions during the collection period, daily meteorological data were obtained from the automatic weather station of the National Meteorological Institute (INMET, Instituto Nacional de Meteorologia), covering January 2016 to December 2018, complemented by a 10-year historical series (2011–2022) for the months of December, January, and February. For January 2017, the mean temperature ranged from 25.6°C to 27.4°C, with maximum temperatures reaching 32.1°C and minimum temperatures between 21.1°C and 24.9°C. The mean relative humidity was 74.5%. The mean temperature in January 2017 (26.6°C) was virtually identical to the historical January mean (26.4°C). The relative humidity (74.5%) was slightly lower than the values recorded in January 2016 (77.6%) and January 2018 (79.1%) but remained within the characteristic range for the region (70–85%). Therefore, the collection occurred under high temperature, moderate-to-high humidity, and conditions typical of the rainy season for this Atlantic Forest remnant.

2.3. Essential Oil Extraction

Fresh leaves were transported to the laboratory immediately after collection and subjected to hydrodistillation via a modified Clevenger apparatus. To ensure reproducibility, all extractions were performed under controlled conditions (constant temperature, pressure, and duration). The yield was calculated on the basis of fresh mass (wet basis) via the following formula: $EO = (V_{EO}/B_m) \times 100$, where EO is the essential oil content (mL per 100 g of fresh biomass), V_{EO} is the volume of essential oil obtained (mL), and B_m is the fresh biomass (g). The extracted oil was stored in amber glass vials at -20°C until analysis.

2.4. Chemical analysis (GC–MS/GC–FID)

Chemical analyses were performed via gas chromatography coupled with mass spectrometry (GC–MS) and gas chromatography with flame ionization detection (GC–FID) at the Analytical Platform of Farmanguinhos, Fiocruz (Rio de Janeiro, Brazil). GC–MS analyses were carried out on an Agilent 6890 chromatograph coupled to an Agilent 5973 N mass selective detector. Both analyses employed an HP-5MS capillary column ($30\text{ m} \times 0.32\text{ mm} \times 0.25\text{ }\mu\text{m}$; Agilent J&W, USA). The oven temperature was programmed from 60 to 240°C at a rate of $3^{\circ}\text{C min}^{-1}$. The injector and detector temperatures were maintained at 250°C , and injections ($1\text{ }\mu\text{L}$) were performed in splitless mode.

The identification of chemical constituents was based on a comparison of mass spectra with the Wiley 7n spectral libraries and a comparison of retention indices (RIs) calculated against literature values [11]. Retention indices were determined via a homologous series of n-alkanes ($\text{C}_8\text{--C}_{28}$). The relative quantification of the compounds was performed via normalization of the peak areas from the GC–FID chromatograms, which are expressed as percentages.

2.5. Cytotoxicity against *Artemia salina*

Cytotoxicity was evaluated via the brine shrimp lethality assay against *Artemia salina* nauplii, adapted from Vanhaecke *et al.* [9] and described by Meyer *et al.* [10]. The *A. salina* assay is used as a preliminary toxicity screening method for natural samples because of its simplicity, low cost, and reproducibility [10]. Cysts of *Artemia salina* (0.2 g) were hatched in artificial saline solution (23 g L^{-1} sea salt and 0.7 g L^{-1} sodium bicarbonate in distilled water) at 25°C under constant illumination. After 48 h, approximately 10 nauplii were transferred to 20 mL plastic wells containing 18 mL of saline solution and 2 mL of test solution. The essential oil was dissolved in DMSO (final concentration of 2%) and tested at concentrations of 0, 10, 20, 30, 40, 50, 100, 250, 500, and $1000\text{ }\mu\text{g mL}^{-1}$. A negative control (2% DMSO in saline solution) was included in all the experiments. Tests were performed in triplicate with two independent replicates. Mortality was recorded after 24 h; immobile individuals were considered dead. Lethal doses were calculated via probit regression via Rbio software. Samples with $\text{LD}_{50} < 1000\text{ }\mu\text{g mL}^{-1}$ were considered biologically active according to the criteria of Meyer *et al.* [10].

2.6. Antibacterial activity determination by disc diffusion

Antibacterial activity was evaluated via the disc diffusion method at the Microbiology Laboratory of the Universidade do Estado da Bahia (UNEB), Campus X, Teixeira de Freitas. Bacterial strains of *Staphylococcus aureus* and *Escherichia coli* were suspended in saline solution (0.9% NaCl) to a turbidity equivalent to the 0.5 McFarland standard and seeded uniformly onto Mueller–Hinton agar plates. Sterile paper discs (6.0 mm diameter) were impregnated with 20 μL of the essential oil at four concentrations, 100%, 50%, 25%, and 12.5% (v/v), prepared by serial dilution in distilled water with 5% DMSO. The controls included a negative disc (5% DMSO) and positive discs (vancomycin for *S. aureus*; chloramphenicol for *E. coli*). The plates were incubated at 37°C for 24 h. The data were analyzed via the nonparametric Kruskal–Wallis test (SAS®). Statistical significance was set at $p < 0.001$.

2.7. *In silico* analyses

The two major constituents of the essential oil, apiole (39.81%) and dillapiole (26.47%), were selected for *in silico* analyses. Structures were confirmed via consultation with PubChem: apiole (CID 10659; SMILES: COC1=C2C(=C(C(=C1)CC=C)OC)OCO2) and dillapiole (CID 10231; SMILES: COC1=C(C2=C(C=C1CC=C)OCO2)OC).

The prediction of the biological activity spectra was performed via the PASS (Prediction of Activity Spectra for Substances) platform (<http://way2drug.com/PASSonline/>), which is based on a Bayesian approach and multilevel neighborhoods of atoms (MNA) descriptors [12]. Activities with $\text{Pa} \geq 0.70$ were considered, as recommended by the method developers [12]. The prediction of acute toxicity in rodents was performed with GUSAR software [13], which estimates LD_{50} values in rats via the intraperitoneal (IP), intravenous (IV), oral, and subcutaneous (SC) routes. The classification followed OECD criteria. Additionally, predictions of specific antibacterial targets were performed via a PASS module trained on ChEMBL data (version 24), comprising 41,065 compounds, with a mean prediction accuracy (IAP = AUC-ROC) of 0.93 [14]. Targets with score = $\text{Pa} - \text{Pi} > 0$ were considered. All analyses were performed between January and February 2026.

3. Results

3.1. Chemical Composition of the Essential Oil

The essential oil obtained from fresh leaves of *P. macedoi* presented a yield of 0.4% (v/w). GC–MS analysis identified 34 compounds distributed into four main chemical classes: monoterpenes (13.53%), sesquiterpenes (13.48%), esters (0.27%), and, predominantly, arylpropanoids (66.28%). The complete chemical composition of this oil, including retention indices and relative percentages of all identified constituents, was previously reported by dos Santos *et al.* [16]. The chemical profile revealed a marked dominance of apiole (39.81%) and dillapiole (26.47%), which together accounted for 66.28% of the total composition. This predominance characterizes the analyzed sample as presenting a profile dominated by apiole and dillapiole. The remaining constituents appear in considerably smaller proportions, with the most relevant being the sesquiterpene β -acoradiene (4.88%) and the monoterpenes *cis*- β -ocimene (4.55%) and β -phellandrene (2.02%).

3.2. Cytotoxicity against *Artemia salina*

The essential oil of *P. macedoi* exhibited high toxicity against *Artemia salina*, with a clear concentration-dependent effect. Mortality rates ranged from 35% at the lowest tested concentration (10 µg·mL⁻¹) to 100% at 1000 µg·mL⁻¹. Probit regression analysis yielded an LD₅₀ of 123.33 µg·mL⁻¹ (95% CI: 93.34–157.15 µg·mL⁻¹), classifying the oil as highly toxic according to the criteria of Meyer *et al.* [10] (LD₅₀ < 1000 µg·mL⁻¹). The dose–response regression curve is shown in Fig. 2.

3.3. Antibacterial activity

The essential oil exhibited antibacterial activity against both strains tested, with differences in response profile (Table 1). Inhibitory halos against *E. coli* were observed at all the tested concentrations, ranging from 10.33 mm (100%) to 5.33 mm (12.5%). The activity against *S. aureus* was restricted to the highest concentrations (100%: 9.33 mm; 50%: 8.33 mm), with no measurable effect at the lower concentrations (25% and 12.5%). The difference in activity was statistically significant ($p < 0.001$, Kruskal–Wallis test).

Table 1
Antibacterial activity of *P. macedoi* essential oil (inhibition halos, mm).

Essential Oil Concentration	<i>Staphylococcus aureus</i> (mm)			Mean (mm)	<i>Escherichia coli</i> (mm)			Mean (mm)
	R1	R2	R3		R1	R2	R3	
100%	8	10	10	9.33	11	10	10	10.33
50%	8	9	8	8.33	10	10	10	10.00
25%	0	0	0	0.00	10	10	9	9.67
12.5%	0	0	0	0.00	6	5	5	5.33
Controle (+)	18	18	18	18.00	31	31	31	31.00
Controle (-)	0	0	0	0.00	0	0	0	0.00

3.4. In silico results

Predictions of acute toxicity in rodents have indicated that apirole and dillapiole exhibit route-dependent toxicity (Table 2). The lowest LD₅₀ values were observed for the intravenous route (38.5 mg·kg⁻¹ for apirole and 39.9 mg·kg⁻¹ for dillapiole; OECD Class 3), whereas the oral route yielded the highest values (2147 mg·kg⁻¹ and 2296 mg·kg⁻¹, respectively; OECD Class 5), suggesting lower relative toxicity upon ingestion.

Table 2
Predicted acute toxicity parameters for apirole and dillapiole in rats (GUSAR).

Compound	Route	log ₁₀ (LD ₅₀ mmol·kg ⁻¹)	LD ₅₀ (mg·kg ⁻¹)	OECD Class
Apirole	IP	0.342 (in AD)	488.5	Class 4
	IV	-0.761 (in AD)	38.5	Class 3
	Oral	0.985 (in AD)	2147.0	Class 5
	SC	0.393 (in AD)	549.9	Class 4
Dillapiole	IP	0.214 (in AD)	364.0	Class 4
	IV	-0.746 (in AD)	39.9	Class 3
	Oral	1.014 (in AD)	2296.0	Class 5
	SC	0.192 (in AD)	345.4	Class 4

AD = within the applicability domain; IP = intraperitoneal; IV = intravenous; SC = subcutaneous.

Predictions of toxicity in target organs (Table 3) indicated that the stomach is the primary organ affected by both compounds across different animal models, with a high probability of occurrence. The liver appears to be an important secondary target, particularly for dillapiole in male rats (Pa = 0.769).

Table 3
Predicted toxicity target organs (Pa ≥ 0.38; high-confidence predictions in bold).

Species	Sex	Organ	Apiole (Pa/Pi)	Dillapiole (Pa/Pi)
Rat	Male	Stomach	0.945/0.017	0.949/0.016
		Liver	0.510/0.170	0.769/0.028
		Kidney	0.480/0.354	–
	Female	Stomach	0.842/0.041	0.790/0.055
		Liver	–	–
Mouse	Male	Stomach	0.847/0.026	0.832/0.034
		Liver	0.525/0.120	0.614/0.094
		Vascular system	0.431/0.174	0.511/0.099
	Female	Liver	0.616/0.090	0.696/0.065
		Stomach	0.388/0.253	0.344/0.320
		Lung	–	0.380/0.315

The major predicted bacterial targets for both compounds are predominantly gram-negative bacteria (Table 4), with emphasis on *Neisseria gonorrhoeae*, *Aeromonas hydrophila*, and *Porphyromonas gingivalis*, providing mechanistic support for the greater experimental effect observed against *E. coli*.

Table 4
Bacterial targets predicted by PASS (score > 0.05).

Compound	Bacterial species	Score	ChEMBL ID
Apiole	<i>Porphyromonas gingivalis</i>	0.1401	CHEMBL614414
	<i>Aeromonas hydrophila</i>	0.1137	CHEMBL612509
	<i>Neisseria gonorrhoeae</i>	0.1072	CHEMBL614430
	<i>Brachyspira hyodysenteriae</i>	0.0778	CHEMBL612491
	<i>Yersinia pestis</i>	0.0586	CHEMBL614597
Dillapiole	<i>Streptococcus</i> sp. group B	0.1665	CHEMBL613186
	<i>Mycobacterium intracellulare</i>	0.1351	CHEMBL612640
	<i>Neisseria gonorrhoeae</i>	0.1244	CHEMBL614430
	<i>Porphyromonas gingivalis</i>	0.1205	CHEMBL614414
	<i>Aeromonas hydrophila</i>	0.1064	CHEMBL612509
	<i>Brachyspira hyodysenteriae</i>	0.0670	CHEMBL612491

Anticancer activity predictions (Table 5) demonstrated that both compounds have moderate-to-high probabilities for several tumor cell lines, with an emphasis on lung carcinomas and melanoma. The high predicted probability of activity in the WI-38 VA13 cell line (nonmalignant cells) indicates low tumor selectivity.

Table 5
Predicted anticancer activity ($P_a \geq 0.50$).

Compound	Cell line	Cancer type	Tissue	P_a	P_i
Apiole	NCI-H322M	Non-small cell lung carcinoma	Lung	0.656	0.009
	NCI-H226	Non-small cell lung carcinoma	Lung	0.643	0.011
	RPML-8226	Multiple myeloma	Hematopoietic	0.588	0.008
	SK-MEL-28	Melanoma	Skin	0.586	0.011
	786-0	Renal carcinoma	Kidney	0.568	0.014
	KM12	Colon adenocarcinoma	Colon	0.534	0.015
	SK-MEL-5	Melanoma	Skin	0.511	0.014
	Hs 683	Oligodendroglioma	Brain	0.526	0.048
	A549	Lung carcinoma	Lung	0.521	0.057
	WI-38 VA13*	Embryonic lung fibroblast	Lung	0.773	0.009
Dillapiole	NCI-H322M	Non-small cell lung carcinoma	Lung	0.651	0.009
	SK-MEL-28	Melanoma	Skin	0.639	0.009
	NCI-H226	Non-small cell lung carcinoma	Lung	0.641	0.012
	A549	Lung carcinoma	Lung	0.631	0.034
	Hs 683	Oligodendroglioma	Brain	0.555	0.038
	SF-268	Glioblastoma	Brain	0.525	0.017
	WI-38 VA13*	Embryonic lung fibroblast	Lung	0.694	0.011

*WI-38 VA13 is an immortalized embryonic lung fibroblast line (nonmalignant).

Predictions of general biological activities (Table 6) indicate that apiole and dillapiole have a high probability of exerting multiple pharmacological functions. Among the most relevant activities are carminative effects ($P_a > 0.94$), caspase-3 stimulation ($P_a > 0.85$), and general anesthetic action ($P_a > 0.81$), in addition to pediculicidal, anti-eczematic, and neurotransmitter reuptake inhibitory activities.

Table 6
Spectrum of biological activities predicted by PASS for apiole and dillapiole ($P_a > 0.70$).

Biological Activity	Apiole (P_a/P_i)	Dillapiole (P_a/P_i)
Anesthetic general	0.880/0.004	0.816/0.004
Antiallergic	0.709/0.008	–
Antieczematic	0.795/0.020	0.751/0.030
Apoptosis agonist	0.739/0.012	0.829/0.006
Aspulvinone dimethylallyltransferase inhibitor	0.832/0.025	0.810/0.031
Carminative	0.946/0.001	0.962/0.001
Caspase 3 stimulant	0.885/0.004	0.856/0.004
CDP-glycerol glycerophosphotransferase inhibitor	0.703/0.053	–
GABA aminotransferase inhibitor	–	0.704/0.004
Membrane integrity agonist	0.822/0.031	0.901/0.011
Neurotransmitter uptake inhibitor	0.712/0.010	0.756/0.006
Ovulation inhibitor	–	0.771/0.004
Pediculicide	0.773/0.002	0.798/0.002
Steroid N-acetylglucosaminyltransferase inhibitor	0.738/0.004	–

– " indicates $P_a < 0.70$ or $P_a < P_i$.

4. Discussion

4.1. Intraspecific Chemical Variation in *P. macedoi*: The Apiole/Dillapiole Profile in Context

The essential oil yield of 0.4% (v/w) obtained from the leaves of *P. macedoi* in this study is consistent with values reported by Fonseca *et al.* [15] and dos Santos *et al.* [16], who also obtained 0.4% for the same species collected from the same locality. The chemical composition revealed marked intraspecific variation: Batista *et al.* [4] identified piperitone (26.48%), silvestrene (13.03%), and bicyclogermacrene (10.45%) as major compounds; Oliveira *et al.* [5] reported trans- β -ocimene ($\approx$ 28%) and sarisan ($\approx$ 17%); and Dias [17] reported profiles dominated by monoterpenes. In contrast, Bezerra *et al.* [6] and Santana *et al.* [7] reported profiles with apiole and dillapiole as major compounds in proportions comparable to those of the present study.

This intraspecific variability is well recognized within the genus *Piper* and may be attributed to factors such as geographic location, altitude, seasonality, phenological stage, and edaphoclimatic conditions. According to the review by Salehi *et al.* [8], the chemical composition of *Piper* essential oils is highly variable both inter- and intraspecifically, and these differences appear to depend on polymorphisms, the plant organ analyzed, geographic differences, environmental conditions, and the existence of distinct chemotypes. Importantly, the apiole/dillapiole-dominated profile observed in the present study was not reproduced in subsequent collections of the same population of the species, indicating that this composition may not represent a stable chemotype but rather transient metabolic expression.

The collection during the rainy season, associated with high temperatures (mean 26.6°C) and high water availability, favors the biosynthesis of phenylpropanoids in *Piper* species. Studies have demonstrated that the composition of *Piper* essential oils varies with seasonality and collection site [3] and that higher temperatures are correlated with greater oil yields [18]. Additionally, compositional variability, including the predominance of phenylpropanoids such as safrole, myristicin, dillapiole, and asarone, is strongly influenced by environmental and geographic factors, giving rise to distinct chemical patterns or context-dependent profiles [19]. For example, Amazonian populations of *Piper aduncum* present high dillapiole content, whereas specimens from southern Brazil do not, suggesting that climatic conditions such as high rainfall and temperature optimize the production of these compounds via the shikimate pathway [20].

From a biosynthetic perspective, the O-methyltransferase enzyme AgOMT1, characterized in *Anethum graveolens*, catalyzes the conversion of hydroxylated precursors with high regioselectivity and is capable of producing both dillapiole (from 6-hydroxymyristicin) and apiole (from 2-hydroxymyristicin) [21]. This complementary enzymatic specificity explains the co-occurrence of apiole and dillapiole in plants of the genus *Piper*, as observed in *Piper permucronatum* (rich in dillapiole and myristicin) and *Piper hostmanianum* (rich in asaricin and myristicin). Thus, the diversity of phenylpropanoids in *P. macedoi* likely reflects the coordinated activity of O-methyltransferases with distinct regioselectivities, which act on precursors with different hydroxylation patterns in response to favorable environmental conditions [3]. Therefore, the chemical composition described here should be interpreted as a context-dependent occurrence rather than a fixed chemotypic classification.

4.2. Cytotoxicity and Mechanisms of Action

The essential oil of *P. macedoi* demonstrated high cytotoxicity against *Artemia salina* ($LD_{50} = 123.33 \mu\text{g}\cdot\text{mL}^{-1}$), classifying it as highly toxic according to the criteria of Meyer *et al.* [10]. This pattern is consistent with findings reported for essential oils of other *Piper* species [22, 23]. Computational predictions by PASS corroborate this activity: apiole and dillapiole exhibited a high probability of acting as apoptosis agonists, caspase-3 stimulants, and membrane integrity agonists. Lien *et al.* [24] demonstrated that apiole induces apoptosis in COLO 205 cells via the activation of caspases 3, 8, and 9, which converges with the predictions of the present study. Ho *et al.* [25] confirmed the antitumor potential of an apiole derivative *in vivo* in colon cancer xenograft models.

Predictions of acute toxicity by the GUSAR indicate moderate-to-low oral toxicity (OECD Class 5; $LD_{50} > 2000 \text{ mg}\cdot\text{kg}^{-1}$) but considerably greater toxicity via the intravenous route (OECD Class 3; $LD_{50} \approx 39 \text{ mg}\cdot\text{kg}^{-1}$). Tabassum *et al.* [26] caution that prolonged ingestion of apiole may cause hepatic and renal toxicity as well as anemia, in accordance with the target organ predictions of the present study. The high-confidence prediction of gastrotoxicity ($Pa > 0.84$ for stomach in rats) apparently contrasts with the gastroprotective effect of dillapiole demonstrated by Rojas-Martínez *et al.* [27] (up to 85.5% protection at $100 \text{ mg}\cdot\text{kg}^{-1}$), suggesting biphasic, concentration-dependent behavior common to many bioactive plant compounds. Additionally, apiole has been shown to inhibit CYP1A1 and CYP1A2 ($IC_{50} = 32.1 \mu\text{M}$ for CYP1A1) [28], indicating its chemoprotective potential and possible drug interactions.

4.3. Antibacterial Activity: Selectivity and Mechanistic Basis

The essential oil demonstrated greater antibacterial efficacy against *E. coli* (gram-negative) than against *S. aureus* (gram-positive), a result that appears counterintuitive given that gram-negative bacteria possess an additional outer membrane that generally confers greater resistance to antimicrobial compounds. However, this membrane is rich in porins that are relatively permeable to amphiphilic molecules such as arylpropanoids [29, 30]. The lipophilic nature of the apiole and dillapiole favors penetration into lipid-rich membranes. The computational predictions reinforce this pattern: all major predicted bacterial targets for apiole and dillapiole are gram-negative (*Neisseria gonorrhoeae*, *Aeromonas hydrophila*, *Porphyromonas gingivalis*, and *Yersinia pestis*), which converges with greater experimental effects against *E. coli*.

Importantly, the disc diffusion method has intrinsic limitations: it does not allow determination of the minimum inhibitory concentration (MIC), nor does it distinguish between bacteriostatic and bactericidal effects. Future studies employing MIC determination via broth microdilution are necessary for more complete characterization.

4.4. Broader Pharmacological Potential and Integrated Perspectives

The *in silico* predictions indicate a considerably broader pharmacological spectrum. Anticancer activity predictions revealed moderate probabilities for several tumor cell lines but elevated predicted activity for the WI-38 VA13 cell line (nonmalignant cells), indicating low tumor selectivity. De Lima *et al.* [31] demonstrated that apiole produces a significant synergistic effect when combined with doxorubicin and vincristine in resistant cell lines (multidrug-resistant;

MDR) through the inhibition of P-glycoprotein, positioning it as a potential chemotherapy adjuvant. The predicted carminative effect (apiole: $P_a = 0.946$; dillapiole: $P_a = 0.962$) is consistent with the ethnobotanical use of *Piper* species for gastrointestinal disorders. A comprehensive review of the genus *Piper* documents the traditional carminative, antifatulent, and digestive uses of several species, including *Piper abbreviatum*, *P. aduncum*, *P. cubeba*, *P. guineense*, *P. longum*, *P. nigrum*, *P. marginatum*, and *P. umbellatum* [8], and the gastroprotective data for dillapiole [27].

The predicted pediculicidal activity ($P_a > 0.77$) is especially relevant when contextualized with the literature: Fazolin *et al.* [32] reported that dillapiole is the most active compound in *Piper aduncum* oil against *Spodoptera frugiperda* ($LD_{50} = 0.35$ ppm); Da Silva *et al.* [33] and Domingos *et al.* [34] reported larvicidal activity of dillapiole derivatives against *Aedes aegypti* and *A. albopictus*; Pereira Filho *et al.* [35] demonstrated acaricidal activity against *Amblyomma sculptum* ($LC_{50} = 3.38$ mg·mL⁻¹); and Bezerra *et al.* [6] reported acaricidal activity of *P. macedoi* oil against *Rhipicephalus sanguineus* with 80.67% mortality at 500 µg·mL⁻¹. The predicted inhibition of GABA aminotransferase activity by dillapiole ($P_a = 0.704$) and the inhibition of neurotransmitter reuptake by both compounds indicate possible activity in the central nervous system. Previous studies have demonstrated that plant phenylpropanoids possess anti-inflammatory and antioxidant properties that modulate molecular pathways associated with neuroprotection and the inflammatory response in human tissues, including the nervous system [36, 37]. These compounds are capable of acting as neuroprotective agents, thus supporting the prediction of their effects on GABAergic transmission and neurotransmitter reuptake.

5. Conclusions

This study demonstrated that the essential oil from the leaves of *P. macedoi* Yunck., collected in the Atlantic Forest of extreme southern Bahia, exhibits an atypical chemical profile dominated by the arylpropanoids apiole (39.81%) and dillapiole (26.47%), which together represent 66.28% of the total composition. This profile represents a specific compositional occurrence, distinct from the majority of reports for the species and convergent with samples from specific localities, suggesting that its expression may be modulated by specific environmental and temporal conditions and may not be consistently reproducible, possibly related to the seasonality of collection (rainy season, austral summer) and the edaphoclimatic conditions of the studied Atlantic Forest fragment.

The dominance of arylpropanoids conferred a coherent and broad bioactive profile on the essential oil. The high cytotoxicity against *Artemia salina* ($LD_{50} = 123.33$ µg·mL⁻¹) and the selective antibacterial activity against *Escherichia coli* are mechanistically aligned with the *in silico* predictions, which indicated that apiole and dillapiole act as apoptosis agonists, caspase-3 stimulants, and inhibitors of predominantly gram-negative bacterial targets. This convergence between experimental and computational data reinforces the internal consistency of the study and establishes a mechanistic basis for the observed activities. Acute toxicity predictions indicate a favorable safety profile via the oral route (OECD Class 5) but greater risk via the intravenous route (OECD Class 3), which should be considered in future preclinical studies.

From a prospective standpoint, the results of this study justify further investigations comprising (i) fractionation of the essential oil and testing of isolated apiole and dillapiole; (ii) MIC determination by broth microdilution for a more complete characterization of antibacterial activity; (iii) cytotoxicity assays in mammalian cell lines to evaluate tumor selectivity; (iv) studies across different collection seasons to elucidate the environmental factors that determine the expression of the apiole/dillapiole-dominated profile; and (v) mechanistic studies focused on the apoptotic and membrane integrity pathways indicated by computational predictions. The data presented contribute to the knowledge of the chemical diversity of the genus *Piper* in the Atlantic Forest and position *P. macedoi* as a promising species for pharmacological investigations and ectoparasite control. The lack of recurrence of this profile in subsequent collections reinforces the importance of longitudinal and multiseasonal studies to better understand the dynamics of secondary metabolism in this species.

Declarations

Funding Declaration

This study was supported by the Federal University of Southern Bahia (UFSB) through an undergraduate research scholarship (Iniciação Científica) granted to one of the authors.

Clinical Trial Number

Not applicable.

Ethics and consent to participate

The plant material (leaves of *Piper macedoi* Yunck.) was collected from a wild population in a remnant of the Atlantic Forest at Fazenda Palmeira, Teixeira de Freitas, Bahia, Brazil. The collection complied with Brazilian local and national guidelines for access to genetic heritage and associated traditional knowledge (Law 13.123/2015 and Decree 8.772/2016). The collection was authorized by the System of Authorization and Information on Biodiversity (SISBIO) under licence number 31544. Appropriate permissions and licences for the collection of plant specimens were obtained from the Brazilian biodiversity authority (SISBIO, licence no. 31544). No further ethical approval was required for this study.

Consent to participate –

Not applicable.

Consent to publish –

Not applicable.

Author Contribution

R.G.D. conceived the study and carried out the majority of the manuscript development, including writing, revision, translation into English, organization of figures and text, as well as performing the chemical analyses, acting as the lead author. Y.S.F. contributed to the microbiological tests and assisted in the revision of parts of the manuscript. J.L.F. contributed to the biological methodology, particularly the bacteriological tests, and participated in the manuscript revision. G.L.O. performed a full review of the manuscript and acted as the research advisor and project coordinator. All authors reviewed and approved the final version of the manuscript.

Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

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Figures

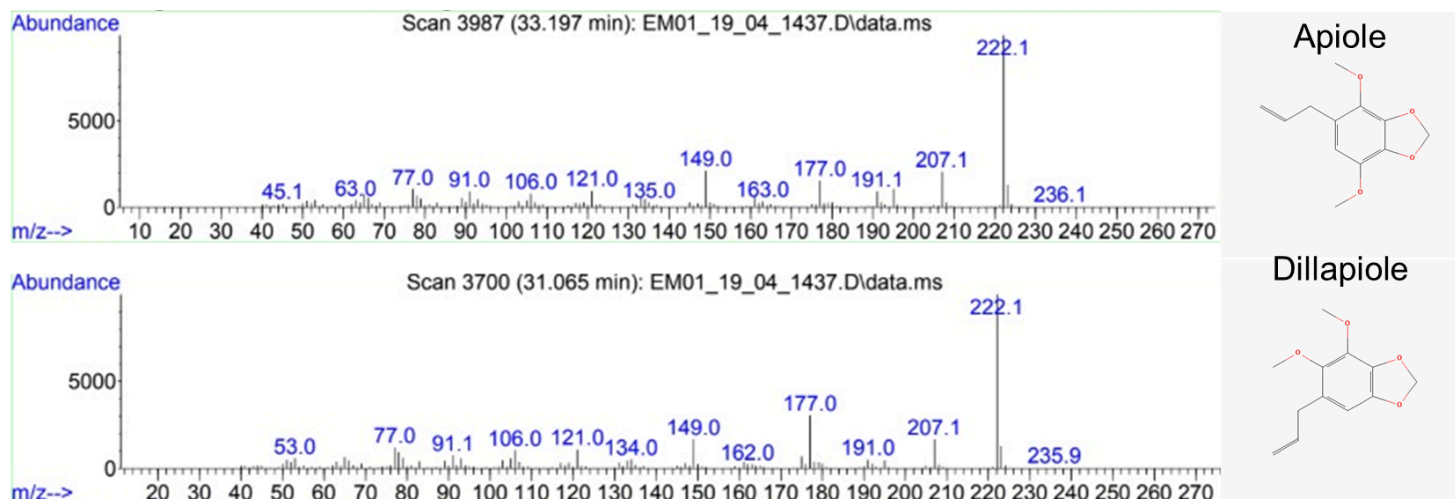


Figure 1

Chromatographic profile and chemical structures of the major compounds in the essential oil of *Piper macedoi* Yunck

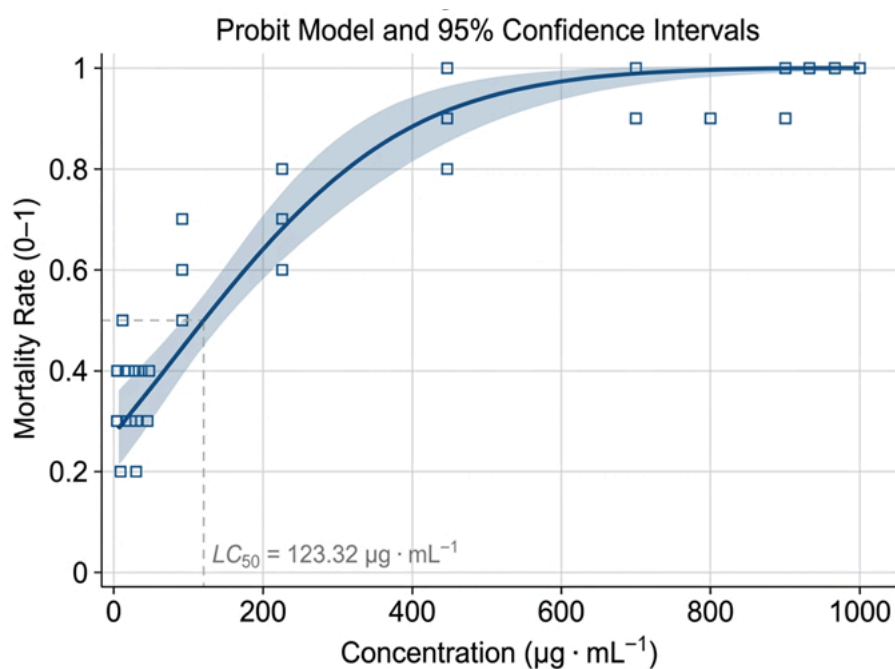


Figure 2

Probit regression curve relating the dose of *P. macedoi* essential oil ($\mu\text{g}/\text{mL}$) to the lethality rate of *Artemia salina* after 24 h of exposure