ACTA
AMAZONICA

http://dx.doi.org/10.1590/1809-4392202303901

CHEMISTRY AND PHARMACOLOGY | ORIGINAL ARTICLE

Acaricidal, insecticidal, acetylcholinesterase, and antioxidant activities of essential oil from *Piper bartlingianum* and *Piper goeldii*

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ABSTRACT

Essential oils derived from species of the genus *Piper* are widely recognized for their diverse chemical profiles and broad spectrum of biological properties, making them promising candidates for various applications in agriculture, pharmacology, and natural product research. This study describes, for the first time, the chemical composition of the essential oil of *Piper goeldii,* and evaluated the antioxidant activity, inhibition of acetylcholinesterase (AChE), and the acaricidal (*Tetranychus urticae*) and insecticidal (*Plutella xylostella*) action of the essential oils from leaves of *P. goeldii* and *P. bartlingianum*. Extraction was performed by hydrodistillation. The oil from *P. bartlingianum* had a greater yield. The major constituents according to a chemical analysis (GC-MS) were *β*-selinene (23.99 ± 0.93%) and *iso*-caryophyllene (20.43 ± 1.11%) for the *P. bartlingianum* oil, and terpinolene (20.18 ± 0.96%) and *β*-caryophyllene (18.23 ± 0.86%) for the *P. goeldii* oil. Irrespective of the agricultural pest, the oil from *P. goeldii* was approximately twice as toxic as the oil from *P. bartlingianum* and also exhibited greater antioxidant activity. The AChE inhibition potential of the *P. bartlingianum* oil was 1.5-fold greater than that of the *P. goeldii* oil. Toxicity tests against non-target organism demonstrated that the oils were not toxic to *Artemia salina*. The results suggest a correlation between acaricidal/insecticidal activity and both DPPH and ABTS radical scavenging in both oils. Our findings indicate that the essential oil from *P. goeldii* has potential for use as an active ingredient in a product for the control of *T. urticae* and *P. xylostella*.

KEYWORDS: chemical composition, *Artemia salina*, DPPH, ABTS, *Tetranychus urticae*, *Plutella xylostella*

Atividade acaricida, inseticida, acetilcolinesterase e antioxidante dos óleos essenciais de *Piper bartlingianum* e *Piper goeldii*

RESUMO

Óleos essenciais derivados de espécies do gênero *Piper* são amplamente reconhecidos na literatura científica por sua diversidade química e amplo espectro de propriedades biológicas, tornando-os candidatos promissores para diversas aplicações na agricultura, farmacologia e pesquisa em produtos naturais. Este estudo descreve, pela primeira vez, a composição química do óleo essencial de *Piper goeldii*, e avaliou a atividade antioxidante, inibição da acetilcolinesterase (AChE) e ação acaricida (*Tetranychus urticae*) e inseticida (*Plutella xylostella*) dos óleos essenciais das folhas de *P. goeldii* e *P. bartlingianum*. Análise química por CG-EM do óleo de *P. bartlingianum* permitiu identificar *β*-selineno (23,99 ± 0,93%) e *iso*-cariofileno (20,43 ± 1,11%) como constituintes majoritários. Por outro lado, terpinolene (20,18 ± 0,96%) e *β*-cariofileno (18,23 ± 0,86%) foram identificados como majoritários no óleo de *P. goeldii*. Independente da praga, o óleo de *P. goeldii* foi cerca de 2 vezes mais tóxico do que o óleo de *P. bartlingianum* e também foi o que apresentou maior atividade antioxidante. O potencial de inibição da enzima AChE do óleo de *P. bartlingianum* foi 1,5 vezes maior do que o óleo de *P. goeldii*. Testes de toxicidade contra organismo não alvo mostraram que os óleos não foram tóxicos a *Artemia salina*. Os resultados obtidos para os óleos de *Piper* sugerem uma correlação entre a atividade acaricida/inseticida and DPPH and ABTS radical scavenging test. Estes resultados sugerem que o óleo de *P. goeldii* é promissor para uso como ingrediente ativo na formulação de um produto para o controle de *T. urticae* e *P. xylostella*.

PALAVRAS-CHAVE: composição química, *Artemia salina*, DPPH, ABTS, *Tetranychus urticae, Plutella xylostella*

CITE AS: Souza, S.C.; Lopes, F.A.M.H.; Monteiro, V.B.; Araujo, C.A.; Camara, C.A.G.; Moraes, M.M.; Vasconcelos, G.J.N.; Pereira, M.R.S.; Zartman, C.E. 2025. Acaricidal, insecticidal, acetylcholinesterase, and antioxidant activities of essential oil from *Piper bartlingianum* and *Piper goeldii. Acta Amazonica* 55: e55cp23390.

INTRODUCTION

Plants of the family Piperaceae stand out among species of potential use for the pharmaceutical industry and agriculture, and are widely distributed in tropical and subtropical regions. In Brazil, 297 species of the genus *Piper* are distributed throughout the country, 185 of which occur in the Amazon region (Guimarães *et al*. 2022).

Species of *Piper* are traditionally used in the treatment of venereal diseases, intestinal problems, epilepsy, and as contraceptive methods (Schultes *et al*. 1975), as well as for the control of arthropods (Schultes and Raffauf 1990). The leaves of *Piper umbellatum*, *P. hispidum*, and *P. auritum*, which occur in the northeastern Brazilian Amazon, are used by indigenous peoples for the prevention of malaria and for combatting lice (Schultes 1980). Overall, however, few species of Amazonian *Piper* have had their phytochemical and biological properties prospected (Morais *et al*. 2007; Santana *et al*. 2022; Araujo *et al*. 2021).

Piper goeldii C.DC., a shrub endemic to the Amazon biome for which no traditional use is known, has not yet been the object of chemical or biological studies. *Piper bartlingianum* (Miq.) C. DC., another shrub native to the northeastern, mid-western and northern regions of Brazil, is traditionally used by fishing communities to temporarily immobilize fish in stream sections, facilitating capture (Acevedo-Rodriguez 1990). The benzene extract and essential oil obtained from the leaves of *P. bartlingianum* collected in the Brazilian Amazon contained the amide dihydropiplartin (Kijjoa *et al*. 1982), as well as sesquiterpenes (Santos *et al*. 1998), as main components, respectively. The biological properties of the essential oil of these species have not yet been studied.

The aim of this study was to determine, for the first time, the chemical composition of the essential oil obtained from the leaves of *P. goeldii*. Additionally, the study evaluated the biological activity of the essential oils from *P. goeldii* and *P. bartlingianum* against two significant agricultural pests, *Tetranychus urticae* Koch and *Plutella xylostella* Linnaeus. The oils were also assessed for their potential as acetylcholinesterase inhibitors, as well as for their antioxidant and cytotoxic effects.

MATERIAL AND METHODS

Collection of plant material

Fresh leaves were collected from three wild specimens of *Piper bartlingianum* at a forest edge in the city of Manaus (02°39'04"S; 60°02'29"W) and from three wild specimens of *Piper goeldii* at the forest edge in the city of Manacapuru (03°11,186'S; 60°26'48"W), in Amazonas state, Brazil. The plants were identified by botanist M.R. Pereira (Instituto Nacional de Pesquisas da Amazônia - INPA) and registered in the Brazilian National System for Management of Genetic Heritage and Associated Traditional Knowledge (*Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado*) (SisGen accession code # A335599). Vouchers of both samples were deposited in the INPA herbarium under numbers INPA 702 (*P. bartlingianum*) and INPA 695 (*P. goeldii*).

Essential oil extraction

The essential oil from fresh leaves (100 g) of the three plants of each species was obtained separately by hydrodistillation in a modified Clevenger-type apparatus for 2 h. The oil layers were separated and dried over anhydrous sodium sulfate, stored in hermetically sealed glass containers, and kept at a low temperature (-5 ºC) until the acaricidal assays and analysis. The oil yields and chemical composition percentages were expressed as the mean ± standard deviations separately for each of the three specimens of each species. Due to the chemical similarity among the three samples within species, we used a single sample of each essential oil per species for all biological essays.

Chemicals

All monoterpenes (β-pinene, ρ-cymene, limonene, 1,8-cineole, and terpinolene) and sesquiterpenes (β-caryophyllene, α-humulene, and spathulenol) were purchased from Sigma-Aldrich, Brazil. The insecticides Azamax®, Decis®, Ortus®, and Lannate®, and the chemotherapy drug cyclophosphamide were used as positive controls in the bioassays and were purchased locally in the city of Recife, Pernambuco, Brazil.

Gas chromatography FID analysis

Quantitative GC-FID analysis was carried out using a Shimadzu Nexis GC-2030 apparatus equipped with a flame ionization detector (FID) and a non-polar DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 µm) (J & W Scientific). The oven temperature was programmed from 60 to 240 °C at a rate of 3°C min^{-1}. Injector and detector temperatures were 260 °C. Hydrogen was used as the carrier gas at a flow rate of 1 mL min^{-1} in split mode (1:30). The injection volume was 0.5 µL of a diluted solution (1/100) of oil in *n*-hexane. The percentage of each compound was obtained from GC-FID peak areas in the order of the DB-5 column elution and expressed as the relative percentage of the area of the chromatograms. Analyses were conducted in triplicate.

Gas chromatography-mass spectrometry analysis

The GC-MS analysis of the essential oil samples was carried out using a Varian 220-MS IT GC system with a mass selective detector, mass spectrometer in EI 70 eV with a scanning interval of 0.5 s and fragments from 40 to 550 Da fitted with the same column and temperature program as for the GC-FID analysis: carrier gas = helium; flow rate = 1 mL min^{-1}; split mode (1:30); injected volume = 1 µL of diluted solution (1/100) of oil in *n*-hexane.

Identification of components

The components were identified based on GC-MS retention indices with reference to a homologous series of C_8-C_{40} *n*-alkanes calculated using the Van Den Dool and Kratz equation (Van Den Dool and Kratz 1963) by computer matching against the mass spectral library of the GC-MS data system (NIST version 21 and WILEY version 21), and co-injection with authentic standards as well as other published mass spectra (Adams 2017). Area percentages were obtained from the GC-FID response without the use of an internal standard or correction factors.

Rearing of *Tetranychus urticae* and *Plutella xylostella*

Specimens of *Tetranychus urticae* (Acari: Tetranychidae) were originally collected from grapevine (*Vitis vinifera* L.) in the municipality of Petrolina, (09º12′43″S; 40º29′12″W), and adult, pupae, larvae, and eggs of *Plutella xylostella* (Lepidoptera: Plutellidae) were originally collected from collard greens (*Brassica oleracea* var. acephala L.) in the city of Recife (08º01'08.3"S, 34º56'45.5"W), both localities in the state of Pernambuco, Brazil. Both organisms were since maintained in the laboratory at the Agronomy Department of Universidade Federal Rural de Pernambuco – UFRPE, reared at a temperature of 25 ± 1 ºC, relative humidity of 65 ± 5%, and a 12-h photoperiod, without any exposure to acaricides or insecticides, respectively. The *T. urticae* breeding method was carried out according to Melo *et al*. (2018). Specimens of *P. xylostella* used for the bioassays were reared on collard greens (*B. oleracea* var. acephala) and the breeding method was carried out according to Bandeira *et al*. (2013).

Residual effect bioassay with *T. urticae*

The leaf disc painting method described by Silva *et al*. (2020) was used to test the action of the essential oils and positive controls by contact toxicity. The experiments were performed with open Petri dishes (10 cm in diameter). Leaf discs (2.5 cm in diameter) were cut from leaves of greenhouse-grown *Canavalia ensiformis* (L.) DC. To determine the appropriate concentrations for the experiments, preliminary tests were conducted using the essential oils at concentrations of 0.01, 1.0, 10, and 100 µg mL^{-1}. Based on the analysis of these initial results, the concentration ranges for further testing were selected. The essential oils of *P. goeldii* and *P. bartlingianum* were tested at concentrations of 1.0, 2.5, 5.0, 10.0, 15.0, 20.0, 25.0, and 30.0 µg mL^{-1} and 1.0, 2.0, 5.0, 10.0, 20.0, 40.0, 60.0, and 70.0 µg mL^{-1}, respectively. The positive control was the broad-spectrum botanical acaricide/insecticide azadirachtin (Azamax® 12 g a.i./L EC, E.I. Parry), tested at concentrations of 0.05, 0.10, 0.15, 0.30, 0.50, 0.60, and 0.70 µg mL^{-1}, and the conventional acaricide fenpyroximate (Ortus® 50 g a.i./L SC, Arysta), tested at concentrations of 0.01, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, and 0.40 µg mL^{-1}. For the negative control, the discs were immersed only in water. The discs were immersed for 30 s in the solutions prepared with essential oil and set to dry on a paper towel at room temperature for 1 min. Each disc was individually placed at the bottom of a Petri dish atop a disc of filter paper (10 cm in diameter) wetted with distilled water. Ten adult female mites were placed into each Petri dish. Mortality was determined under a dissecting microscope 24 h after treatment. Mites were considered dead if the appendages did not move when prodded with a fine paintbrush. All treatments were replicated three times. To estimate the curve slopes and lethal concentration for 50% of the population (LC_{50}) with a 95% confidence level, mortality data from each treatment and control were submitted to PROBIT analysis using the SAS software (version 9.0).

Larvicidal effect bioassay with *P. xylostella*

The residual effect bioassays were based on the method described by Silva *et al*. (2020). The experiments were performed with open Petri dishes (10 cm in diameter). Leaf discs (2.5 cm in diameter) cut from collard greens were immersed for 30 s in solutions prepared with the essential oil and positive controls diluted in aqueous solution and set to dry on a paper towel at room temperature for 1 min. To determine the appropriate concentrations for the experiments, preliminary tests were conducted using the essential oils at concentrations of 0.01, 1.0, 10, and 100 µg mL^{-1}. Based on the analysis of these initial results, the optimal concentration ranges for further testing were selected. The essential oils of *P. goeldii* and *P. bartlingianum* were tested at concentrations of 5.0, 10.0, 15.0, 20.0, 30.0, 40.0, 45.0 and 50.0 µg mL^{-1} and 10.0, 20.0, 30.0, 40.0, 60.0, 70.0, 80.0 and 100.0 µg mL^{-1}, respectively. The positive control was the broad-spectrum botanical acaricide/insecticide azadirachtin (Azamax® 12 g a.i/L EC E.I. Parry), tested at concentrations of 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 and conventional insecticide deltramethrin (Decis® 25 g active ingredient/L EC Bayer CorpScience) tested at concentrations of 10.0, 20.0, 30.0, 40.0, 60.0, 70.0, 80.0 and 100.0 µg mL^{-1}. Negative control disks were immersed only in 1.0% polyoxyethylene sorbitan monolaurate + 0.1% dodecylbenzene sulfonic acid. After drying, the discs were transferred to Petri dishes containing filter paper slightly moistened with distilled water. Thirty instar *P. xylostella* larvae were placed in each dish. Mortality was recorded after 48 h of exposure. The experimental design was entirely randomized, with four repetitions, totaling 120 larvae per treatment and control. To estimate the curve slopes and LC_{50} with a 95% confidence level, mortality data from each treatment and control were submitted to PROBIT analysis using the SAS software (version 9.0).

Acetilcolinesterase inhibition

The inhibition of acetylcholinesterase (AChE) was determined following the colorimetric method described by Ellman (1961). Solutions containing 50.0 µL of phosphate buffer (50 mM), pH 7.4, 125.0 µL of dithionitrobenzoic acid (DTNB, 3 mM

in phosphate buffer, pH 7.4, 25.0 µL of acetylcholinesterase (electric eel AChE 1 U/mL in phosphate buffer, pH 7.4), and 25.0 µL of essential oil diluted in MeOH were added to a 96-well microplate and incubated for 15 min at 25 °C. Next, 25.0 µL of acetylthiocholine iodide (ACTI 15 mM in phosphate buffer, pH 7.4) were added. AChE activity was analyzed in an ELISA EZ Read 2000 microplate reader with absorbance at 405 nm every 13 seconds five times. The essential oils and positive control were analyzed at concentrations of 400 to 0.80 µg mL^{-1}. Lannate® (methomyl), which is an insecticide of the carbamate class that has acetylcholinesterase inhibitors as its mode of action, was used as a positive control. The negative control was only MeOH, without the oil. Each treatment and control was tested in triplicate, that is, each concentration of the oils, independently prepared, as well as the negative and positive controls, was added separately into three wells of a 96-well microplate. After normalization of the data, a nonlinear regression curve was created using the GraphPad Prism v7.01 statistical program.

Toxicity against *Artemia salina*

The brine shrimp, *Artemia salina* Leach was selected to determine the effect of the essential oils in a fast, low-cost bioassay involving a non-target organism for the assessment of the toxicity of a botanical insecticide/acaricide with potential use for the control of agricultural pests. The lethality bioassay was performed following the procedure described by Melo *et al*. (2012). The growth medium was prepared with filtered sea water in a small tank divided into two compartments (one covered and one open). Shrimp eggs were added to the covered compartment and a lamp was placed above the open compartment to attract the hatched shrimp through perforations in the partition wall. After 48 h, the hatchlings matured as nauplii and were used in the assay. Stock test solutions were made by dissolving the essential oil in Tween 80® 0.5% (v/v) and seawater was added to complete 5 mL of the total volume. Appropriate volumes were then added to tubes with seawater containing 10 nauplii to enable different concentrations of essential oil and the positive control. The concentrations of *Piper goeldii* and *Piper bartlingianum* oils ranged from 50.0 to 650.0 µg mL^{-1} (50.0, 100.0, 200.0, 400.0, 500.0, 600.0 and 650.0 µg mL^{-1}) and 50.0 to 600.0 µg mL^{-1} (50.0, 100.0, 200.0, 400.0, 500.0 and 600.0 µg mL^{-1}), respectively. Cyclophosphamide was used as positive control at concentrations ranging from 2 to 45 µg mL^{-1} (2.0, 5.0, 10.0, 20.0, 30.0, 40.0 and 45.0 µg mL^{-1}). Negative control samples contained sea water and Tween 80®. Each treatment and control was tested in triplicate. After 24 h of incubation under light, the number of dead and surviving brine shrimp in each tube was counted. Mortality data obtained from each treatment and control were submitted to Probit analysis using the SAS software (version 9.0) for the determination of the LC_{50} with a 95% confidence level. The toxicity grade of the essential oil was ascribed according to the criteria by Dolabela (1997): LC_{50} values below 80 µg mL^{-1} were considered highly toxic; values between 80 and 250 µg mL^{-1} were regarded as moderately toxic; and values exceeding 250 µg mL^{-1} were classified as non-toxic.

Antioxidant assay

DPPH˙ radical scavenging - The antioxidant activity of the essential oil of the leaves of the two *Piper* species was determined using the free radical DPPH, following the method described by Araujo *et al*. (2021). Stock solutions were prepared from the essential oils at different concentrations (0.10 to 5.0 µg mL^{-1}). After a preliminary analysis, appropriate quantities of stock solutions of the essential oil and 450 µL of the solution of DPPH˙ (23.6 mg mL^{-1} in EtOH) were transferred to 0.5-mL Eppendorf tubes and the volume was completed with EtOH, following homogenization. Samples were sonicated for 30 min and the amount of DPPH˙ was recorded in a UV-vis device (Biochrom EZ Read 2000) at a wavelength of 517 nm in a 96-well plate. The negative control was only MeOH, without the oil. The scavenging of the DPPH free radicals was expressed as the mean effective concentration (EC_{50}) (the concentration of antioxidant required to reduce the original amount of the radicals by 50%). The lower the EC_{50} value, the better the antioxidant potential. Ascorbic acid was used as the positive control. Each treatment and control was tested in triplicate, that is, each concentration of the oil, independently prepared, as well as the negative and positive controls, was added separately into three wells of a 96-well microplate.

ABTS˙⁺ radical cation - The antioxidant activity from the essential oil of the leaves of the two *Piper* species was determined with the radical cation ABTS˙⁺, following the method described by Araujo *et al*. (2021) in a UV-vis device (Biochrom EZ Read 2000), using Trolox as the standard compound. The initial concentrations of the essential oil solutions were 1.0 to 400.0 µg mL^{-1}, with the addition of 450 µL of the radical ABTS˙⁺ solution to give final concentrations of 2.5 to 100.0 µg mL^{-1}. The negative control was only MeOH, without the oil. The positive control was 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). The solutions were protected from light and sonicated for 6 min. The different concentrations of oils, negative control (MeOH) and positive control (Trolox) were distributed in a 96-well microplate and the absorbance was measured at a wavelength of 734 nm. The scavenging of ABTS˙⁺ was expressed as the mean effective concentration (EC_{50}). Each treatment and control was tested in triplicate, that is, each concentration of the oils, as well as the controls, was added separately into three wells of the 96-well microplate. Antiradical efficiency was established using linear regression analysis with a 95% confidence interval ($p < 0.05$) using the GraphPad Prism 5.0 statistical program.

RESULTS

Hydrodistillation of the leaves of both species yielded yellowish oils with a citric aroma. Yields were 0.28 ± 0.02% for *P. bartlingianum* and 0.18 ± 0.01% for *P. goeldii*. The chemical analysis by GC-MS enabled the identification of 43 compounds in the oils, 28 in the *P. bartlingianum* oil and 24 in the *P. goeldii* oil, corresponding to 97.30 ± 1.16% and 96.38 ± 1.08% of the chemical composition of the oils, respectively (Figure 1; Table 1).

The *P. goeldii* oil was mainly composed of monoterpenes (23.58 ± 0.97%) and sesquiterpenes (72.80 ± 0.90%). The major constituents were terpinolene (20.18 ± 0.96%), *β*-caryophyllene (18.23 ± 0.86%), (*Z*)-*α*-*trans*-bergamotol (10.78 ± 0.36%), and spathulenol (9.15 ± 0.22%). The oil from *P. bartlingianum* was mainly composed of sesquiterpenes (96.84 ± 1.16%). *β*-selinene (23.99 ± 0.93%), *iso*-caryophyllene (20.43 ± 1.11%), *β*-caryophyllene (9.40 ± 0.18%), *cis*-sesquisabinene hydrate (7.25 ± 0.26%), and *β*-vetivenene (7.79 ± 0.34%) (Table 1).

Toxicity differed between the oils, the test organism *T. urticae* being more susceptible to both oils than *P. xylostella* (Table 2). Irrespective of the agricultural pest, the oil from *P. goeldii* was approximately twice as toxic than that from *P. bartlingianum*. Neither of the two oils was more toxic to *T. uritcae* than the commercial acaricide used as positive control. However, the essential oil of *P. goeldii* proved to be more toxic to *P. xylostella* than the positive control. When compared to the positive control, the oils of *P. goeldii* and *P. bartlingianum* did not exhibit toxicity to the non-target organism (*A. salina*).

The AChE inhibition potential of the *P. bartlingianum* oil was 1.5-fold greater than that of the *P. goeldii* oil, but 5.4 times lower than that of the positive control Lannate® (Table 3). The antioxidant capacity of *P. goeldii* oil, as evaluated by the DPPH and ABTS assays, was nearly twice as high as that of *P. bartlingianum* oil in both tests. However, its activity was significantly lower compared to the positive controls used in the study (Table 3).

DISCUSSION

A similar yield for the essential oil from the leaves of *P; bartlingianum* was reported from the municipality of Porto Velho in the state of Rondônia (northern Brazil) (Santos *et al*. 1998). The yield of the essential oil from other *Piper* species from the region of Manaus was also similar to what we obtained for *P. bartlingianum* (Morais *et al*. 2007; Rameshkumar *et al*. 2011; Araújo *et al*. 2018).

While *β*-caryophyllene was present in the essential oil of *P. bartlingianum* from Manaus (this study) and Rondônia (Santos *et al*. 1998) in proportions <10%, the compounds with the highest proportions in Manaus (*β*-selinene and (Z)-caryophyllene) were not detected in Rondônia. In turn, the major constituents of the *P. bartlingianum* oil in Rondônia,

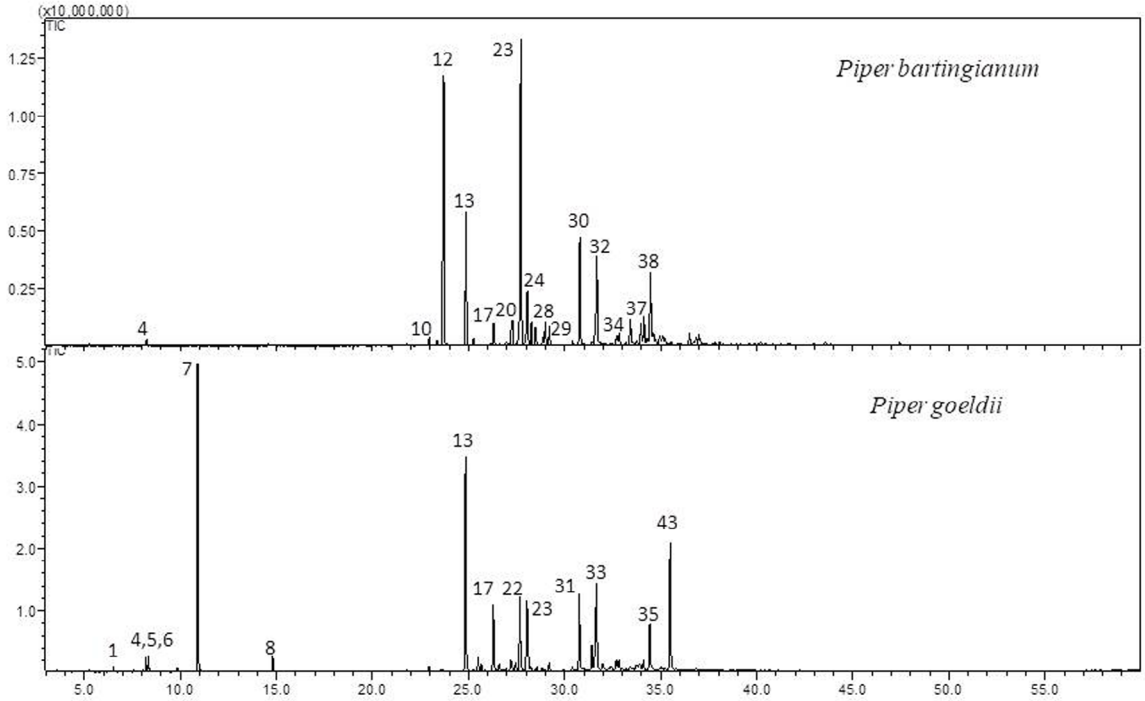

Figure 1. GC-MS chromatogram of the essential oil of *Piper bartlingianum* and *Piper goeldii* leaves. The number of each peak in the chromatogram corresponds to the number displayed in Table 1.

Table 1. Chemical composition of the essential oil from leaves of *Piper bartlingianum* and *Piper goeldii*. The relative percentage of each compound obtained from GC-FID peak areas is presented in the order of the DB-5 column elution (Nr.). Values are the mean ± standard deviation based on three plants of each species.

Nr.	Compound	RI[a]	RI[b]	*P. bartlingianum* (%)	*P. goeldii* (%)	Identification method
1	*β*-pinene	972	966	0.04 ± 0.00	0.26 ± 0.02	RI, MS,CI
2	*δ*-3-carene	1005	1008	0.03 ± 0.00	-	RI, MS
3	*ρ*-cymene	1015	1020	0.07 ± 0.00	-	RI, MS,CI
4	Limonene	1024	1022	0.32 ± 0.02	0.88 ± 0.09	RI, MS,CI
5	1,8-cineole	1031	1028	-	0.97 ± 0.05	RI, MS,CI
6	*cis*-linalool oxide	1067	1065	-	0.28 ± 0.01	RI, MS
7	Terpinolene	1088	1082	-	20.18 ± 0.96	RI, MS,CI
8	*trans*-*β*-terpineol	1159	1156	-	1.01 ± 0.10	RI, MS
9	*α*-cubebene	1348	1344	-	0.10 ± 0.00	RI, MS
10	*α*-copaene	1376	1371	0.48 ± 0.01	0.40 ± 0.06	RI, MS,CI
11	*β*-elemene	1384	1389	0.36 ± 0.01	-	RI, MS
12	*iso*-caryophyllene	1406	1408	20.43 ± 1.11	-	RI, MS
13	*β*-caryophyllene	1417	1419	9.40 ± 0.18	18.23 ± 0.86	RI, MS,CI
14	*β*-gurjunene	1432	1431	0.48 ± 0.02	-	RI, MS
15	*α*-*trans*-bergamotene	1432	1433	-	0.99 ± 0.08	RI, MS
16	*α*-guaiene	1439	1441	-	0.47 ± 0.03	RI, MS
17	*α*-humulene	1452	1455	1.43 ± 0.08	5.39 ± 0.18	RI, MS,CI
18	*allo*-aromadendrene	1458	1460	-	0.44 ± 0.02	RI, MS
19	4,5-di-*epi*-aristolochene	1473	1476	-	0.17 ± 0.01	RI, MS
20	*γ*-muurolene	1479	1478	2.67 ± 0.09	-	RI, MS
21	*β*-chamigrene	1477	1480	-	0.98 ± 0.03	RI, MS
22	*γ*-himachalene	1481	1483	-	6.66 ± 0.21	RI, MS
23	*β*-selinene	1489	1493	23.99 ± 0.93	6.72 ± 0.15	RI, MS
24	*δ*-selinene	1495	1492	4.11 ± 0.12	-	RI, MS
25	*α*-muurolene	1502	1500	1.57 ± 0.07	-	RI, MS
26	Germacrene A	1510	1508	1.10 ± 0.13	-	RI, MS
27	*δ*-amorphene	1511	1515	0.36 ± 0.00	0.27 ± 0.02	RI, MS
28	7-*epi*-*α*-selinene	1520	1520	1.52 ± 0.05	-	RI, MS
29	*trans*-calamenene	1521	1526	1.26 ± 0.06	0.29 ± 0.01	RI, MS
30	*cis*-sesquisabinene hydrate	1544	1542	7.25 ± 0.26	-	RI, MS
31	Elemol	1549	1553	-	5.63 ± 0.11	RI, MS
32	*β*-vetivenene	1554	1554	7.79 ± 0.34	2.12 ± 0.05	RI, MS
33	Spathulenol	1577	1583	-	9.15 ± 0.22	RI, MS,CI
34	1-*epi*-cubenol	1630	1627	1.78 ± 0.08	-	RI, MS
35	*γ*-eudesmol	1630	1636	-	4.01 ± 0.21	RI, MS
36	*α*-muurolol	1648	1644	1.46 ± 0.00	-	RI, MS
37	Cubenol	1650	1645	1.97 ± 0.00	-	RI, MS
38	Pogostol	1654	1651	5.49 ± 0.18	-	RI, MS
39	*cis*-calamenen-10-ol	1664	1660	0.42 ± 0.05	-	RI, MS
40	*trans*-calamenen-10-ol	1672	1668	0.44 ± 0.01	-	RI, MS
41	Cadalene	1677	1675	0.76 ± 0.06	-	RI, MS
42	Khusinol	1680	1677	0.32 ± 0.02	-	RI, MS
43	(Z)-*α*-*trans*-bergamotol	1690	1696	-	10.78 ± 0.36	RI, MS
	Total			97.30	96.38	
	Monoterpenes			0.46	23.58	
	Sesquiterpenes			96.84	72.80	

Nr = order of elution of chemical constituents identified in a 30-m DB-5 capillary column; RI = retention index; a Retention index calculated from retention times in relation to those of a series of n-alkanes in a 30-m DB-5 capillary column; b Linear retention indices from the literature. SD = standard deviation, MS = mass spectrum, CI = co-injection with authentic standards.

Table 2. Toxicity of essential oils from leaves of *Piper bartlingianum* and *Piper goeldii* against *Tetranychus urticae* and *Plutella xylostella* by residual contact and toxicity to a non-target organism (*Artemia salina*). N = total number of organisms tested; LC_{50}95% CI) = estimated mean lethal concentration (µg mL^{-1}) and its 95% confidence interval. Slope ± SE = mean linear coefficient of the dose-response curve and its standard error; χ^2 (DF) = chi-square test statistic and its degree of freedom ($P > 0.05$).

Essential oil/ positive control	N	LC_{50} (95% CI)	Slope ± SE	χ^2 (DF)
P. *goeldii*	718	14.66 (11.51-17.82)	2.06 ± 0.36	5.55 (6)
P. bartlingianum	900	33.16 (29.39-36.79)	4.50 ± 0.47	6.18 (8)
Azamax®	630	0.30 (0.26-0.37)	2.45 ± 0.14	8.33 (5)
Ortus®	810	0.14 (0.10-0.18)	1.00 ± 0.07	8.70 (7)
P. goeldii	630	26.94 (21.01-31.02)	1.11 ± 0.12	4.15 (5)
P. bartlingianum	630	56.25 (50.47-59.31)	5.10 ± 0.65	5.25 (5)
Azamax®	630	4.89 (3.60-6.58)	0.91 ± 0.08	4.83 (5)
*Decis®	640	40.36 (34.22–45.15)	2.30 ± 0.13	6.18 (5)
P. goeldii	940	326.94 (321.12-341.44)	3.23 ± 0.17	6.34 (7)
P. bartlingianum	940	286.25 (264.43-299.22)	4.10 ± 0.88	7.65 (7)
Cyclophosphamide	940	19.7 (17.3-22.1)	3.97 ± 0.80	2.83 (7)

Table 3. Inhibition concentration (IC_{50}) of acetylcholinesterase (AChE) and antioxidant activity (EC_{50}) by DPPH and ABTS tests of *Piper bartlingianum and Piper goeldii* essential oils and positive controls. Values are presented as mean ± standard deviation (n = 3).

Essential oil/ positive control	AChE IC_{50} (µg mL^{-1})	DPPH EC_{50} (µg mL^{-1})	ABTS EC_{50} (µg mL^{-1})
P. *bartlingianum*	19.2 ± 0.6	227.6 ± 8.1	219.0 ± 6.1
P. *goeldii*	28.9 ± 1.1	127.9 ± 4.8	112.2 ± 5.9
Lannate®	3.5 ± 0.9	-	-
Ascorbic acid	-	5.6 ± 0.5	-
Trolox	-	5.5 ± 0.5	4.1 ± 0.8

the sesquiterpenes *β*-elemene (10.5%) and (*E*)-nerolidol (9.0%) (Santos *et al*. 1998) were not detected in our study. Kijjoa *et al*. (1982) reported the presence of dihydroplatin amide in the benzene extract of *P. bartlingianum* leaves collected by the Transamazonian Highway. However, in the present study, no nitrogenous compounds were detected. Differences in the chemical composition of the essential oil of a same species among collection sites may be explained by different geographic and climatic conditions, as well as genetic variability, which influence the production and accumulation of chemical constituents (da Camara *et al*. 2017).

No previous reports on the chemical composition of the essential oil of *P. goeldii* were found in the literature. Terpinolene (20.18 ± 0.96%), the main constituent of the *P. goeldii* oil, was also reported in proportions between 10 and 20% in *Piper obliquum* Ruiz & Pav. (Guerrini *et al*. 2009), *Piper corcovadensis* (Miq.) C.DC. (Silva *et al*. 2016), and *Piper hispidinervum* C.DC. (Filho *et al*. 2010).

This is the first report of acaricidal and insecticidal activity of essential oils from the leaves of *P. bartlingianum* and *P. goeldii*. The greater toxicity of the *P. goeldii* oil against the two tested arthropods may be attributed to the qualitative and quantitative differences in the chemical composition of the essential oils (Moraes *et al*. 2012). There are several reports on the effect of *Piper* essential oils on *T. urticae* and *P. xylostella*. The oil of *P. aduncum* L. (LC_{50} = 5.83 µg ml^{-1}) from the state of Pernambuco (northeastern Brazil) (Araujo *et al*. 2020a) was more toxic to *T. urticae* than our *Piper* oils, which, in turn, were 2.5- to sixfold more toxic to *T. urticae* than the oil of *Piper hispidinervum* C. DC. from Manaus (Silva *et al*. 2019). The essential oil of *P. capitarianum* Yunck (LC_{50} = 0.21 µg mL^{-1}) and *P. krukoffi* Yunck (LC_{50} = 0.37 µg mL^{-1}) (Santana *et al*. 2022) were much more toxic to *P. xylostella* than our oils, which, in turn, were 37 to 78 times more toxic to *P. xylostella* than the extract of the fruit of *P. aduncum* from Indonesia (Ningsih *et al*. 2020). These differences in toxicity among *Piper* congeners may be related to the type of matrix studied, the chemical derivative (extract or essential oil), as well as qualitative and quantitative differences in the constituents of the essential oils (Araujo *et al*. 2020b; Santana *et al*. 2022).

There are few published studies on the toxicity of plant-derived substances to non-target organisms when the aim is to create a natural product as an alternative to conventional insecticides. The mean lethal concentration against the non-target *A. salina* obtained in our study suggests that the essential oils from *P. bartlingianum* and *P. goeldii* may be environmentally safe at concentrations that proved efficient in the tests with *T. urticae* and *P. xylostella*. However, to assess the environmental impact of these essential oils, it is necessary to conduct tests on other non-target organisms, such as pollinators, freshwater fish, natural enemies of pests, vertebrates, and other ecologically relevant species.

The AChE inhibition test provides information on the mode of action by which a mixture of chemical constituents such as in essential oils exerts biological effects on other organisms (Coban *et al*. 2016). Substances that inhibit the enzyme AChE promote hyperactivation, altered motor reflexes, respiration spasms, ataxia, and tremors, and can lead to the death of mammals and insects, depending on the degree of poisoning (Coban *et al*. 2016). Our results suggest that the essential oil from *P. bartlingianum* contains a higher concentration of substances that inhibit AChE activity compared to *P. goeldii*. Consequently, it is likely to induce higher mortality in both target and non-target organisms than the essential oil from *P. goeldii*.

The better antioxidant activity of the oil from *P. goeldii* may be explained by the presence of oxygenated constituents, such as elemol, spathulenol, *γ*-eudesmol, and (*Z*)-*α*-*trans*-bergamotol, which are known for their antioxidant properties (El-Gawad 2016; Nogueira Neto *et al*. 2013). Our results for antioxidant activity are similar to those reported for the

essential oil from *Piper brachypetiolatum* Yunck (64.8 µg ml^{-1}) and *P. madeiranum* Yunck (66.8 µg ml^{-1}), which also occur in the region of Manaus (Araujo *et al*. 2021).

CONCLUSIONS

This is the first report of the chemical composition of the essential oil of *Piper goeldii*. Both the essential oil of *P. goeldii* and *P. bartlingianum* collected in the central Brazilian Amazon, were rich in sesquiterpenes. Our results suggest that the essential oils from *P. goeldii* and *P. bartlingianum* (especially the former) are promising as active ingredients in a product for the control of *T. urticae* and *P. xylostella*. Further studies are needed to establish the cost-benefit ratio for use in organic farming and to ascertain the environmental safety for other types of non-target organisms.

ACKNOWLEDGMENTS

The authors are grateful to Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (APQ-1033-1.06/21), Conselho Nacional de Desenvolvimento Científico e Tecnológico (PQ-2 306514/2022-2), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for awarding a grant (PNPD-88887.474239/2020-00) and Laboratório Multiusuário de Análises do Departamento de Química at Universidade Federal Rural de Pernambuco for the chromatographic analysis.

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RECEIVED: 07/02/2024
ACCEPTED: 30/01/2025
ASSOCIATE EDITOR: Jorge Mauricio David
DATA AVAILABILITY: The data that support the findings of this study are available, upon reasonable request, from the corresponding author, Claudio Augusto Gomes da Camara.