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Influence of drying on the chemical composition and bioactivity of *Piper aduncum* (Piperaceae) essential oil against *Aedes aegypti* (*Diptera: Culicidae*)

Influência da secagem na composição química e bioatividade do óleo essencial de de *Piper aduncum* (Piperaceae) sobre *Aedes aegypti* (*Diptera: Culicidae*)

Influencia del secado en la composición química y bioactividad del aceite esencial de *Piper aduncum* (Piperaceae) en *Aedes aegypti* (*Diptera: Culicidae*)

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Abstract
Piper species are producers of essential oils with high yield and promising chemical composition for both perfumery and the pharmaceutical industry. They present bioactivity against pathogens and against insect pests, whether agricultural or medical, such as *Aedes aegypti*, for example, a vector of arboviruses with a high incidence in tropical and subtropical regions. In this study, an investigation was carried out to elucidate the chemical composition of essential oils from the leaves and inflorescences of *Piper aduncum* collected in the state of Goiás, Brazil. Evaluating the interference of the drying process on yield, chemical composition and larvicide potential against *Ae. aegypti*. Leaves and inflorescences of *P. aduncum* were collected in the rural area of the municipality of Iporá-GO. Fresh and dried samples were processed separately and subjected to hydrodistillation for two hours. The oil obtained was qualitatively evaluated by gas-coupled chromatography and mass spectrometry. Greater yield was observed in samples submitted to the drying process. Oils obtained from fresh samples had a higher percentage of monoterpene hydrocarbons. Variation was observed between the major components of samples of fresh leaves and inflorescences, with eupatoriochrome being the major component in dried samples. Larvicidal activity against *Ae. aegypti* was considered promising (LC50<100µg/mL) in all samples. The results obtained showed a chemical composition different from that generally presented by *P. aduncum*. This reinforces the idea of intraspecific variability of essential oils and the need for chemical evaluation between samples even if they belong to the same species.
Keywords: Bioinsecticide; Vector control; Eupatoriochrome.

Resumo
Espécies de *Piper* são produtoras de óleos essenciais com alto rendimento e com composição química promissora tanto para a perfumaria quanto para a indústria farmacêutica. Apresentam bioatividade contra patógenos e também contra insetos-praga, seja de ordem agrícola ou médica, como o *Aedes aegypti*, por exemplo, vetor de arboviroses com alta incidência as regiões tropicais e subtropicais. Nesse estudo foi realizada uma investigação para elucidar a composição química dos óleos essenciais das folhas e inflorescências de *Piper aduncum* coletadas no estado de Goiás, Brasil. Avaliando a interferência do processo de secagem no rendimento, na composição química e no potencial larvicida contra *Ae. aegypti*. Folhas e inflorescências de *P. aduncum* foram coletados na zona rural do município de Iporá-GO. Amostras frescas e desidratadas foram processadas separadamente e submetidas a hisdrodestilação por duas horas. O óleo obtido foi avaliado qualitativamente por Cromatografia a Gás acoplada e Espectrometria de Massas. Observou-se maior rendimento nas amostras submetidas ao processo de desidratação. Os óleos obtidos de amostras frescas apresentaram maior porcentagem de hidrocarbonetos monoterpenos. Observou-se variação entre os componentes majoritários de amostras de folhas e inflorescências frescas, sendo o eupatoriocromeno majoritário nas amostras desidratadas. A atividade larvicida contra *Ae. aegypti* foi considerada promissora (CL50<100μg/mL) em todas as amostras. Os resultados obtidos mostraram uma composição química diferente da geralmente apresentada por *P. aduncum*. Isso reforça a ideia de variabilidade intraespecífica dos óleos essenciais e a necessidade da avaliação química entre amostras ainda que pertençam a uma mesma espécie.
Palavras-chave: Bioinseticida; Controle de vetores; Eupatoriocromeno.

Resumen
Las especies de *Piper* son productoras de aceites esenciales con alto rendimiento y una composición química prometedora tanto para la perfumería como para la industria farmacéutica. Presentan bioactividad frente a patógenos y también frente a plagas de insectos, ya sean agrícolas o médicos, como *Aedes aegypti*, por ejemplo, vector de arbovirus con alta incidencia en regiones tropicales y subtropicales. En este estudio se realizó una investigación para dilucidar la composición química de los aceites esenciales de las hojas e inflorescencias de *Piper aduncum* recolectadas en el estado de Goiás, Brasil. Evaluar la interferencia del proceso de secado sobre el rendimiento, la composición química y el potencial larvicida frente a Ae. aegypti. Se recolectaron hojas e inflorescencias de *P. aduncum* en el área rural del municipio de Iporá-GO. Las muestras frescas y deshidratadas se procesaron por separado y se sometieron a hidrodestilación durante dos horas. El aceite obtenido se evaluó cualitativamente mediante cromatografía de gas acoplado y espectrometría de masas. Se observó mayor rendimiento en las muestras sometidas al proceso de deshidratación. Los aceites obtenidos de muestras frescas tenían un mayor porcentaje de hidrocarburos monoterpénicos. Se observó variación entre los componentes principales de las muestras de hojas frescas e inflorescencias, siendo el eupatoriocromo el componente principal en las muestras deshidratadas. Actividad larvicida contra *Ae. aegypti* se consideró prometedor (CL50 <100μg/mL) en todas las muestras. Los resultados obtenidos mostraron una composición química diferente a la que generalmente presenta *P. aduncum*. Esto refuerza la idea de la variabilidad intraespecífica de los aceites esenciales y la necesidad de una evaluación química entre muestras, incluso si pertenecen a la misma especie.
Palabras clave: Biopesticida; Control de vectores; Eupatoriochromene.

1. Introduction

The family Piperaceae is represented by plants of herbaceous, shrub sizes or small trees (Gogosz *et al*., 2012), with specimens distributed in tropical and subtropical regions of the planet (Judd *et al*., 2009). They are part of the group of angiosperms, which generally present in their phytochemical composition alkaloids, cardiac glycosides, coumarins, flavonoids, saponins, and triterpenes associated with different biological and pharmacological activities (Albuquerque *et al*., 2020). The genus *Piper* is considered the largest genus of the family, encompassing more than 700 described species, of which approximately 170 are native to Brazilian biomes (Sousa et al, 2008). Distinct species of *Piper* are used for medicinal purposes as anti-inflammatory, anxiolytic, anticonvulsant, sedative, antidiarrheal, and in urinary disorders (Oliveira *et al*., 2012).

Piperaceans are also essential oils producers. Some constituents common to essential oils of *Piper* are: safrole, predominant in the species *Piper hispidinervum*; ɣ-Terpinene and ρ-Cymene in *Piper marginatum*; safrole and dillapiole in *Piper aduncum* (Pereira Filho *et al.*, 2021; Dos-Santos *et al*., 2018; Star *et al*., 2006). These constituents are known to be bioactive against bacteria, fungi, protozoa, and insects (Carballo-Arce *et al*., 2019). Dillapiole for example showed insecticidal potential against agricultural pests such as *Sitophilus zeamais* (Coleoptera: Curculionidae), *Spodoptera frugiperda*, and *Helicoverpa armigera* (Lepidoptera: Noctuidae), and urban pests such as *Aedes aegypti* (Diptera: Culicidae) (Durofil *et al*., 2021; Morais *et al*., 2007; Estrela *et al*., 2006). This constituent is a potent inhibitor of cytochrome activity (P450 and CYP34A), acting synergistically with pyrethroids and causing insect mortality including strains resistant to current insecticides (Carballo-Arce *et al*., 2019). Inhibition of these cytochromes in the insect reduces the ability to excrete xenobiotics present in food, where the death of the insect occurs through the accumulation of toxic substances located in the digestive tract (Li *et al*., 2007).

Essential oils are produced by aromatic plants for the purpose of protecting the species against various environmental parameters. The use of the term "essential" for volatile oils occurs because they have odorous components in their composition (Simões, 2017). In general, volatile oils have very unstable characteristics, especially in the presence of light, air, heat, moisture, and metals (Simões, 2010). According to the material acquisition condition, qualitative and quantitative variation is a parameter to be analyzed. In addition to genetic, phenological, edaphic, and climatic factors (Gobbo-Neto & Lopes, 2007; Morais, 2009), the chemical composition of essential oils can be variable depending on the extraction technique and factors such as the time and weather condition at the moment of collection, as well as the processing conditions of the plant material before extraction. These factors can modify the chemical profile of the oil obtained, modify the biosynthetic pathway of the plant, stimulating or inhibiting the production of compounds, as well as interfering with the yield of the process (Costa *et al*., 2005).

P. aduncum has the essential oil with the highest percentage of dillapiole and, therefore, is a promising species for the research and development of bioinseticides (Gainza *et al*., 2016). It is popularly known as monkey-pepper, long pepper, false jaborandi. It is distributed throughout Latin America, from sea level to high altitudes (Fazolin *et al*., 2006). Medicinal use of the plant is reported in popular preparations as a digestive stimulant, sedative, diuretic, antidiarrheal, antimalarial agent, and even as an insect repellent (Pohlit et al 2006). There are many studies involving the biopotential of *P. aduncum* in different Brazilian biomes and in other countries of the American continent.

This research aimed to evaluate the chemical composition of essential oil of leaves and inflorescences of *P. aduncum* from Cerrado of Goiás (Goiás savanna), comparing samples that went through the drying process with fresh samples and the bioactivity of these oils against larvae of *Ae. aegypti* to investigate possible variations in both chemical composition and insecticide potential, depending on the variables observed in the acquisition process.

2. Methodology

2.1 Botanical material harvesting and obtaining the essential oil

Samples of *P. aduncum* were obtained in the rural area of the municipality of Iporá-GO (6°29'45.96"S and 51°10'46.87"O – 828 m) in the morning period in the first half of March 2021, end of the wet season. The region is climatically classified as humid subtropical (Alves & Biudes, 2008). Leaves and inflorescences of various shrubs of the same locality were collected. Botanical material was identified by the biologist Camila Aline Romano, MSc. In the laboratory, the samples were sanitized and screened. Part of the material was dehydrated in a forced air convection oven at 37°C for 24 hours. Another part was immediately undergone to the extraction process.

To obtain the essential oil, fresh and dehydrated leaves and inflorescences were crushed in a conventional blender and subjected to hydrodistillation in a Clevenger apparatus for two hours (Farmacopeia Brasileira, 2019). The essential oil obtained was desiccated with sodium sulfate anhydrous and stored in amber vials under refrigeration at -22°C for further chemical evaluation and bioassays.

2.2 Gas chromatography-mass spectrometry (GC-MS)

Essential oil of *P. aduncum* was subjected to gas chromatography-mass spectrometry (GC-MS) in a Shimadzu GC-

MS QP2010A chromatograph equipped with a DB-5 fused-silica capillary column (30 m × 0.25 mm ID × 0.25 µm and 5%-Phenyl-Methylpolysiloxane) and ramp programmed as follows: 60-240°C at 3°C/min, then at 280°C at 10°C/min, ending with 10min at 280°C. Helium was used as a carrier with a flow of 1mL/min and the injection port at 225°C. Operating conditions of the mass spectrometer were: interface temperature 240°C; electron ionization at 70 eV with scanning mass range of 40-350 m/z and sampling of 1 scan/s. Constituents were identified by comparing their retention indices (Dool; Krstz, 1963) to n-alkanes C_9-C_{26} and mass spectra with data from the literature (ADAMS 2007) and digital library (NIST, 1998).

2.3 Larvicidal activity against *Aedes aegypti*

The essential oils of fresh leaves, dehydrated leaves, fresh inflorescences, and dehydrated inflorescences were tested against third instar larvae of *Ae. aegypti*. Bioassays were performed in a heated chamber with temperature of 28°C±1°C, relative humidity of 85%±5% and photoperiod of approximately 12 hours. An aliquot of essential oils was solubilized with surfactant polysorbate 80 (v/v) and distilled water to produce a solution at a concentration of 100 µg/mL, with which test solutions were prepared in serial dilutions of 100 to 10 µg/mL. A total of 20 larvae were exposed to the test solutions. A solution of water and surfactant was used as negative control, temephos solution (Abate® - Basf Chemical Company) at 0,012 µg/mL was used as a positive control. For each bioassay, three replicates were performed at various times (WHO, 2005).

Mortality events were verified after 24 hours of exposure. The larvae that did not respond to the mechanical stimulation and that presented blackening of the body and the cephalic capsule were considered dead. The data obtained in the larvicide assays were submitted to the nonlinear regression method of *Probit* to determine the LC of 50 and 90% mortality. Analyses were performed with the software *Statistica* Version 12.0 (StatSoft 2013).

3. Results and Discussion

The extractive process resulted in essential oils of yellowish color, and gently sweetish odor. The yield of the extractive process varied between 0.219% for fresh samples and 0.886% for dehydrated samples. Chromatographic analysis revealed 50 substances, presented in Table 1. There was variability in the composition of essential oils that went through the drying process. Samples of fresh hydrodistillated leaves and inflorescences showed a higher percentage of monoterpene hydrocarbons. On the other hand, dehydrated leaves and inflorescences presented a higher percentage of sesquiterpene hydrocarbons (Figure 1).

The extractive process showed higher yield in dehydrated samples. However, in general, the samples had lower yield than that observed in the literature. The samples of this study were collected during rainy periods. Souza et al. (2018) found lower yield values in the extractive process of essential oil of *Spiranthera odoratissima* obtained in Cerrado of Goiás (Goiás savanna). Lemos et al. (2012) observed the variation in the composition of essential oil of *Melaleuca alternifolia* subjected to different drying temperatures, suggesting that the increase in temperature favors the oxidation of monoterpenes such as α-Pinene, as well as the volatilization of these compounds.

Majority compounds of the essential oil of fresh leaves of *P. aduncum* were sarisam (17.81%), E-β-Ocimene (15.57%), myristicin, and piperitone (7.99%). For samples that went through the drying process were found eupatoriochromene (26.76%), germacrene D (14.88%), (E,E)-α-Farnesene (13.68%), δ-Cadinene (6.71%) and β-Caryophyllene (6.57%). In fresh and dehydrated samples of inflorescence were found piperitone (30.46%), terpinen-4-ol (15.15%), limonene (7.77%) and β-Terpinene (7.77%), and again eupatoriochromene (30.51%), β-Caryophyllene (8.85%), α-Ylangene (7.35%) and (E,E)-α-Farnesene (6.29%). Dillapiole was detected only in fresh-extracted leaf samples (5.67%) and inflorescences (1.51%). Valadares et al (2018) evaluated the chemical composition of fresh-extracted essential oils of *P. aduncum*, where piperitone, myristicin, β-Phellandrene, and germacrene D were also majority.

Oliveira et al. (2012) found significant variation in the chemical composition of essential oil of *P. aduncum* from the north of Minas Gerais, where the majority compound was p 1,8-Cineole. In samples originating from Bolivia, the majority component was 1,8-Cineole, occurring in more than 40% of the samples. The Bolivian sample presented approximately 13% phenylpropanoids in its composition (Vila *et al*., 2005). When Maia et al. (1998) investigated the chemical composition of *P. aduncum* collected in different locations in the Amazon region of Brazil, they showed that the oil has a high yield (2.5 to 3.5%) and presence of the phenylpropanoid dillapiole as the majority component, presenting a variation between 30-97% among the samples evaluated. The present study showed a different chemical composition, to those already present in the literature. Differences in the components of the oil probably resulted from different environmental conditions for the development of the plant (Simas *et al*., 2004).

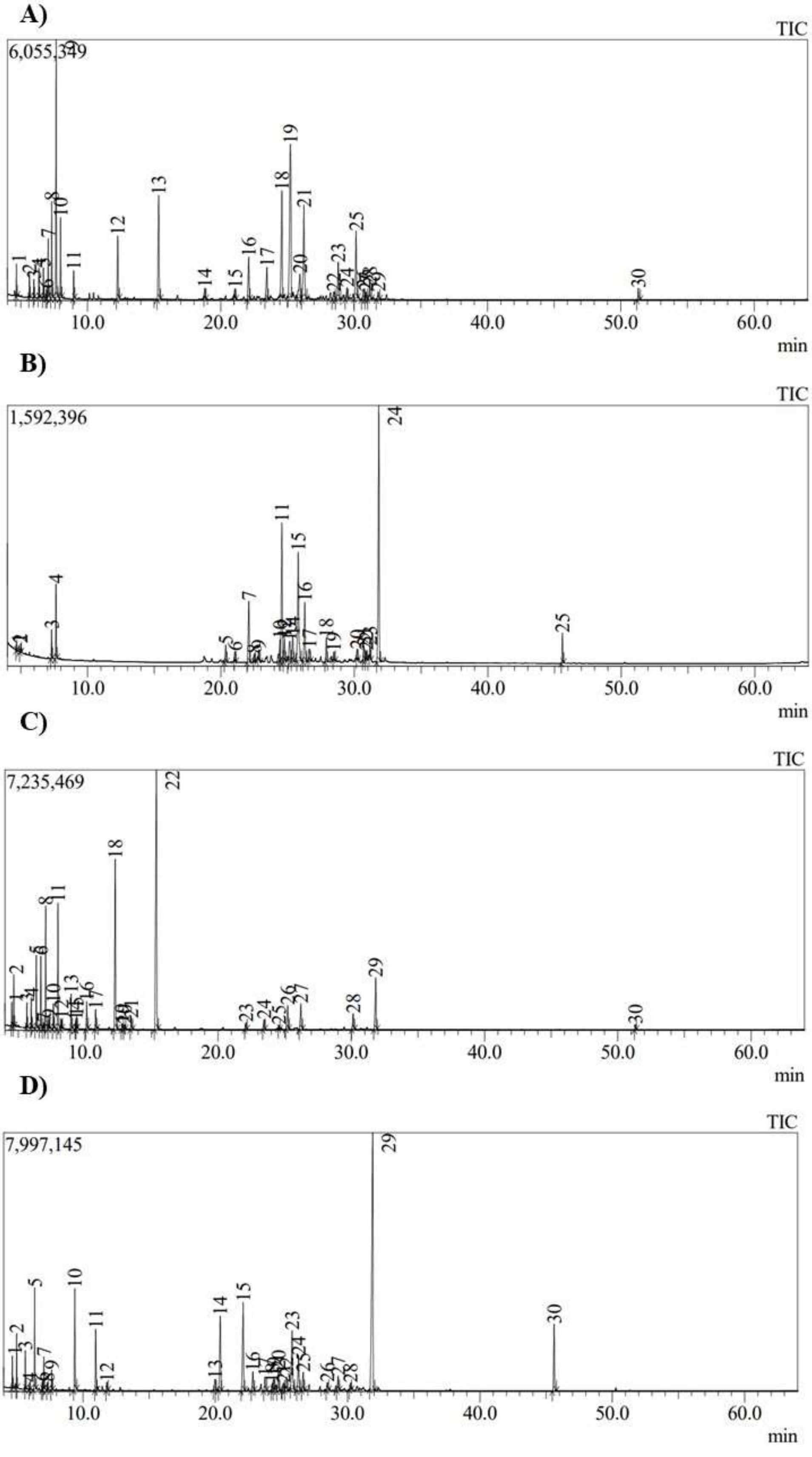
Santana et al. (2015), investigated the composition of essential oil of some species of *Piper*. In the study the main components found in *Piper arboreum* were germacrene D (31.83%) and bicyclogermacrene (21.40%). For *Piper marginatum* the majority were (E)-Methyl isoeugenol (27.08%), (E)-Anethole (23.98%) and (Z)-Methyl isoeugenol (12.01%). They still found (E)-Isocroweacin (29.52%), apiol (28.62%) and elemicin (7.82%) in samples of *P. aduncum* (Santana et al, 2015). Eupatoriochromene, a chromene highly found in *P. aduncum* (Taher et al, 2020) was detected in all samples, being the majority in dehydrated samples of leaves and inflorescences. This compound is bioactive against microorganisms and insects (Taher et al, 2020; Torres et al, 2017). Studies carried out a few decades ago already showed the larvicidal potential of eupatoriochromene on culicides, especially linked to the ability of interfering in the hormonal regulation of immatures and in the process of ecdysis (Klocke et al, 1985; Proksch & Rodrígues, 1983).

Table 1: Chemical constituents and yield of the extractive process of essential oil from leaves and inflorescences of *Piper aduncum.*

KI	Substance	Samples			
		Fresh leaves (%)	Dehydrated eaves (%)	Fresh inflorescences (%)	Dehydrated inflorescences (%)
Monoterpenic hydrocarbons		**39.65**	**6.29**	**79.22**	**22.74**
930	α-thujene				1.28
939	α-Pinene	0.91	0.25	1.91	1.08
954	Camphene		0.34		1.9
979	β-Pinene	0.75		1.08	1.46
990	Myrcene	0.6		1.32	0.27
1002	α-Phellandrene	1.01		3.64	4.61
1017	α-Terpinene	1.12		3.83	
1024	ρ-Cymene	0.32		0.33	0.35
1026	Limonene			7.77	0.35
1030	Silvestrene	2.55			
1034	E-β-Ocimene	15.57	5.7	2	1.33
1059	ɣ-Terpinene	3.51			
1059	β-Terpinene			7.77	
1088	ρ-Mentha-2,4(8)-diene	1.29		2.17	
1096	Linalool			0.82	5.98
1146	Camphor				4.13
1177	Terpinen-4-ol	4.03		15.15	
1208	Piperitol			0.97	
1252	Piperitone	7.99		30.46	
Sesquiterpene hydrocarbons		**57.5**	**88.87**	**14.27**	**67.25**
1338	δ-Elemeno	0.62			
1371	Cyclosativene				0.56
1375	α-Ylangene		1.75		7.35
1390	β-Elemeno	0.43	0.83		
1419	β-Caryophyllene	3.51	6.57	0.66	8.85
1422	β-Copaene		0.61		
1434	α-trans-Bergamotene		0.79		1.81
1446	α-Caryophyllene			1.02	
1454	α-Humulene	2.65			
1456	Farnesene				1.2
1479	ɣ-Muurolene		1.98		0.62
1481	Germacrene D	8.66	14.88	0.29	1.22
1495	Sarisan	17.81		2.7	
1496	Viridiflorene		2.67		0.66
1500	α-Muurolene		3.05		1.62
1505	(E,E)-α-Farnesene		13.68		6.29
1513	ɣ-Cadinene	2.8			
1518	Myristicin	8.85		2.87	
1523	δ-Cadinene		6.71		3.59
1531	(E)-ɣ-Bisabolene		1.41		1.79
1563	(E)-nerolidol		2.28		
1583	Caryophyllene oxide		0.81		0.66
1583	Viridiflorol	3.23			
1600	Humulene epoxide	0.87			
1620	Dillapiole	5.67		1.51	
1628	1-epi-Cubenol		0.85		0.52
1640	Epi-α-Cadinol	1.13	0.8		
1646	α-Muurolol	0.59			
1646	Cubenol		1.49		
1654	Cadinol		0.95		
1758	Eupatoriochromene	0.68	26.76	5.22	30.51
Total		**97.15**	**95.16**	**93.49**	**89.99**
Yield		0.219%	0.511%	0.565%	0.886%

Source: Authors.

Figure 1: Chromatograms of essential oil chemical analysis of leaves and inflorescences of *Piper aduncum*. A) fresh leaves; B) dehydrated leaves; C) fresh inflorescences and D) dehydrated inflorescences.



Source: Authors.

In the bioassays for investigation of larvicidal activity all samples showed promising larvicidal activity (LC_{50}<100 µg/mL) (Table 2). Although fresh leaf essential oil had a lethal effect in lower concentrations, there was no significant difference between the lethal concentrations obtained through the bioassay. Thus, it can be proposed that the oils from dehydrated leaves are more promising because they present bioactivity like the other samples tested, however present higher yield.

Table 2: Bioactivity of essential oil of leaves and inflorescences of *Piper aduncum* against larvae of third instar of *Aedes aegypti*.

	Lethal concentrations (± confidence interval)	
Substance	**LC_{50}**	**LC_{90}**
Fresh leaves	24.5 µg/mL (22.9-26.1 µg/mL)	44.8 µg/mL (42.7-46.9 µg/mL)
Dehydrated eaves	31.54 µg/mL (30.55-32.52 µg/mL)	57.08 µg/mL (58.66-55.50 µg/mL)
Dried inflorescences	34.0 µg/mL (33.5-34.4 µg/mL)	53.1 µg/mL (52.3-53.8 µg/mL)
Dehydrated inflorescences	31.9 µg/mL (29.0-34.8 µg/mL)	45.4 µg/mL (41.0-49.8 µg/mL)

Source: Authors.

In studies of chemical composition of essential oil of Piperaceae species against larvae of *Ae. aegypti*, the main components identified were β-Pinene (32.7%) and β-Caryophyllene (17.1%) in *P. aduncum*. In *P. marginatum* β-Caryophyllene (24.2%) and caryophyllene oxide (20.1%) were the majority. In this study the larvicidal activity presented LC_{50}= 8.29 µg/mL (Costa *et al*., 2010). Piperaceans have several bioactive compounds with insecticidal activity. With respect to *Ae. aegypti,* Marques and Kaplan (2017) reviewed more than 30 compounds with larvicidal potential isolated from extracts and essential oils of *Piper,* in particular, *P. nigrum*, *Piper guineense* and *Piper tuberculatum*, where it was possible to observe the presence of the substances dillapiole and eupatoriochromene. Albuquerque et al. (2020) observed larvicidal activity of *Piper dilatatum* and *Piper hostmannianum* with LC_{50} =97.6 and 93.2 µg/mL, respectively. Pereira Filho *et al.*, (2021) obtained LC_{50} ranging between 45 and 52 ppm against *Ae. aegypti* Rockefeller strain and pyrethroid-resistant isolates respectively.

Oliveira et al. (2012) also investigated the larvicidal activity of essential oil of *P. aduncum*. The authors found values of LC_{50}=289.9 µg / mL, concentration higher than that considered promising and when evaluated 1,8-Cineole, they did not observe a lethal effect. Studies with the essential oil of fruits of *P. aduncum* presented LC_{50}= 30.29 µg/mL, where the composition revealed β-Pinene and limonene as majority compounds (Costa *et al*., 2010). In the present study, the essential oil of the inflorescences of *P. aduncum* presented limonene in its composition. All investigated oils presented as majority compounds with larvicidal activity investigated and proven in previous studies, such as germacrene D (Lima *et al.*, 2019) and the eupatoriochromene (Torres *et al.*, 2017). Thus, although dillapiole is not present in abundant content in some samples, bioactivity remained promising, due to the action of other substances with equal potential, or the synergism and enhancement that the composition can promote.

4. Conclusion

The present study investigated the effect of drying on the chemical composition of essential oil of leaves and inflorescences of *P. aduncum* obtained in the Midwest of Goiás, in addition to its bioactivity against larvae of *Ae. aegypti*. The drying process proved to be efficient to increase the yield of the extractive process in relation to the fresh one. It was also possible to observe that the amount of monoterpenes in the dehydrated samples was lower, due to their volatilization. The chemical composition of essential oils was relatively different from most studies with *P. aduncum* where dillapiole and safrole are majority constituents. Despite the differences in the composition of the oils, the larvicidal bioactivity remained in promising concentrations, mainly because in all samples there is a predominance of substances with already known insecticidal potential, such as eupatoriochromene and germacrene D. These results reinforce the need to evaluate the chemical constitution of essential oils whenever the conditions of collection, processing and extraction vary.

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