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 *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*)

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 monoterpenic 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.
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 contra *Ae. aegypti*. Folhas e inflorescências de *P. aduncum* foram coletados no estado rural do município de Iporá-GO. Amostras 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 hidrocarbonetos monoterpenos. Se observó variación entre los componentes mayoritarios de las muestras de hojas y inflorescencias frescas, siendo el eupatoriocromeno el componente más present en todas las muestras. Los resultados obtenidos mostraron una composición química diferente de la que generalmente presenta *P. aduncum*. Esto reforzó la idea de la variabilidad intraspecífica de los aceites esenciales y la necesidad de una evaluación química entre muestras aún que pertenecen a una misma especie.

Palabras clave: Bioinsecticida; Control de vectores; Eupatoriocromeno.

1. Introducción

El género *Piper* es el más grande del género, incluyendo más de 700 especies descritas, de las cuales aproximadamente 170 son nativas de biomas brasileños (Sousa et al. 2008). Distintas especies de *Piper* se usan para fines medicinales como anti-inflamatorios, ansiolíticos, anticonvulsivos, sedativos, antidiarreicos, y en enfermedades urinarias (Oliveira et al., 2012).

Los aceites esenciales de *Piper* son producidos en grandes cantidades y su composición química puede variar significativamente. Algunas especies como *P. aduncum* (Pereira Filho et al., 2021; Dos-Santos et al., 2018; Star et al., 2006) se han utilizado por su actividad insecticida contra *A. aegypti*. Estos aceites esenciales se usan por su efectividad para controlar plagas de insectos, como *Sitophilus zeamais* (Coleóptera: Curculionidae), *Spodoptera frugiperda*, y *Helicoverpa armigera* (Lepidóptera: Noctuidae), y en agricultura como antibacterianos, fungicidas, y pesticidas.
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 bioinsecticides (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 x 0.25 mm ID x 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 C₉-C₂₆ 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. 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-Terpine (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 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 dillapiolace 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%), apiole (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 ecysis (Klocke *et al*., 1985; Proksch & Rodrigues, 1983).
Table 1: Chemical constituents and yield of the extractive process of essential oil from leaves and inflorescences of *Piper aduncum*.

| KI   | Substance                        | Fresh leaves (%) | Dehydrated eaves (%) | Fresh inflorescences (%) | Dehydrated inflorescences (%) |
|------|----------------------------------|------------------|----------------------|--------------------------|-------------------------------|
|      | Monoterpenic hydrocarbons        |                  |                      |                          |                               |
| 930  | α-thujene                        | 39.65            | 6.29                 | 79.22                    | 22.74                         |
| 939  | α-Pinene                         | 0.91             | 0.25                 | 1.91                     | 1.08                          |
| 954  | Camphene                         | 0.34             |                      |                          |                               |
| 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 | γ-Terpine                        | 3.51             |                      |                          |                               |
| 1059 | β-Terpine                        |                  |                      |                          |                               |
| 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 | 68.87                            | 14.27            |                      | 67.25                    |                               |
| 1338 | δ-Elemeno                        | 0.62             |                      |                          | 0.56                          |
| 1371 | Cyclosativen                      |                  |                      |                          | 7.35                          |
| 1375 | α-Ylangene                       | 1.75             |                      |                          |                               |
| 1390 | β-Elemeno                        | 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 | θ-Murolene                       |                  |                      | 1.98                     | 0.62                          |
| 1481 | Germacrene D                     | 8.66             | 14.88                | 0.29                     | 1.22                          |
| 1495 | Sarisan                          | 17.81            |                      | 2.7                      |                               |
| 1496 | Viridiflorene                    |                  |                      |                          | 0.66                          |
| 1500 | α-Murolene                       |                  |                      | 3.05                     | 1.62                          |
| 1505 | (E,E)-α-Farnesene                |                  |                      | 13.68                    | 6.29                          |
| 1513 | γ-Cadinene                       | 2.8              |                      |                          |                               |
| 1518 | Myristicin                       | 8.85             |                      | 2.87                     |                               |
| 1523 | δ-Cadinene                       |                  |                      | 6.71                     | 3.59                          |
| 1531 | (E)-γ-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 | l-epi-Cubenol                    | 0.85             |                      | 0.85                     | 0.52                          |
| 1640 | Epi-α-Cadinol                    | 1.13             | 0.8                   |                          | 1.79                          |
| 1646 | α-Murolol                        | 0.59             |                      |                          |                               |
| 1646 | Cubenol                          |                  |                      | 1.49                     |                               |
| 1654 | Cadinol                          |                  |                      | 0.95                     |                               |
| 1758 | Eupatoriocromene                 | 0.68             | 26.76                | 5.22                     | 30.51                         |
|      | Total                             |                  |                      |                          |                               |
| 97.15 | 95.16                           | 93.49            |                      | 89.99                    |                               |
|      | Yield                             | 0.219%           | 0.511%               | 0.563%                   | 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 ($\text{LC}_{50}<100\ \mu g/mL$) (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*.

| Substance                  | Lethal concentrations (± confidence interval) |
|----------------------------|-----------------------------------------------|
|                            | $\text{LC}_{50}$                                   | $\text{LC}_{90}$                        |
| Fresh leaves               | 24.5 $\mu g/mL$ (22.9-26.1 $\mu g/mL$)           | 44.8 $\mu g/mL$ (42.7-46.9 $\mu g/mL$) |
| Dehydrated leaves          | 31.54 $\mu g/mL$ (30.55-32.52 $\mu g/mL$)       | 57.08 $\mu g/mL$ (58.66-55.50 $\mu g/mL$) |
| Dried inflorescences       | 34.0 $\mu g/mL$ (33.5-34.4 $\mu g/mL$)           | 53.1 $\mu g/mL$ (52.3-53.8 $\mu g/mL$) |
| Dehydrated inflorescences  | 31.9 $\mu g/mL$ (29.0-34.8 $\mu g/mL$)           | 45.4 $\mu g/mL$ (41.0-49.8 $\mu g/mL$) |

Source: Authors.

In studies of chemical composition of essential oil of Piperaceae species against larvae of *Ae. aegypti*, the main components identified were $\beta$-Pinene (32.7%) and $\beta$-Caryophyllene (17.1%) in *P. aduncum*. In *P. marginatum* $\beta$-Caryophyllene (24.2%) and caryophyllene oxide (20.1%) were the majority. In this study the larvicidal activity presented $\text{LC}_{50}=8.29\ \mu g/mL$ (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 $\text{LC}_{50}=97.6$ and 93.2 $\mu g/mL$, respectively. Pereira Filho et al., (2021) obtained $\text{LC}_{50}$ 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 $\text{LC}_{50}=289.9\ \mu g / mL$, 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 $\text{LC}_{50}= 30.29\ \mu g/mL$, where the composition revealed $\beta$-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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References

Adams, R. P. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, (4a ed.), Allured Publishing Corporation, Illinonis, 2007.

Albuquerque, J. S. Gama, E., França, L. P., & De-Jesus, R. P. (2020). Atividade larvicida dos óleos essenciais de Piper dilatatum e Piper hostmannianum (Piperaceae) para o controle de Aedes aegypti (Culicidae) em laboratório. DiCieência em foco, 4(1), 22-28.

Alves E. D. L., & Biudes, M. (2008). Variabilidade temporal da precipitação em Ipórra, GO – Um estudo climatológico. Revista de Ciências Ambientais, 4: 1-9.

Carballo-Arce, A. F., Raina, V., Liu, S., Liu, R., Jackie, W., Carranza, D., Amason, J. T., & Durst, T. 2019. Potent CYP3A4 inhibitors derived from dillapiol and sesamol. ACS Omega, 4, 10915–10920.

Costa, J. G. M., et al. (2010). Composição e toxicidade de óleos essenciais de espécies de Piper frente a larvas de Aedes aegypti L. (Diptera Culicidae). Latin American Journal of Pharmacy, 29(3), 463-467.

Costa, J. G. M., Rodrigues, F. F. G., Angelico, E. C., Silva, M. R., Mota, M. L., Santos, N. K. A., Cardoso, A. L. H., & Lemos, T. L. G. (2005). Chemical-biological study of the essential oils of Hyptis martiusi, Lippia sidoides and Sicygium aromaticum against larvae of Aedes aegypti and Culex quinquefaciatus. Brazilian Journal of Pharmacognosy, 15(4), 304-309.

Costa, L. C. (2005). Secagem e fragmentação da matéria seca no rendimento e composição do óleo essencial de capim-limão. Horticulura Brasileira, 23(2), 956-959.

Dool, H. V. D., & Kratz, P. D. (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of Chromatograph A.

Fazolin, M., Estrela, J. L. V., Catoni, V., & Costa, C. R. (2006). Potencialidades da pimenta-de-macaco (Piper aduncum L.): características gerais e resultados de pesquisa. Rio Branco, AO: Embrapa Arroz, 46p.

Gainza, Y. A., Fantatto, R. R., Chaves, F. C. M., Bizzo, H. R., Esteves, S. N., & Chagas, A. C. S. (2016). Piper aduncum against Haemonchus contortus isolates: cross resistance the research of natural bioactive compounds. Brazilian Journal of Veterinary Parasitology, 25(4), 383-393.

Gogosz, M. A. et al. (2012). Anatomia foliar comparativa de nove espécies do gênero Piper (Piperaceae), Rodriguésia, 63(2): 405-417.

Judd, W. S., Campbell, C. S., Kellogg, E. A., Stevens, P. S., & Donoghue, M. J. (2009). Sistemática Vegetal: um enfoque filogenético. (3a ed.), Arthem.

Klocke, J. A., Balandrin, M. F., Adams, R. P., & Kingsford, E. (1985). Insecticidal chromenes from the volatile oil of Hemiziona fitchii. Journal of Chemical Ecology, 11(6): 701-712.

Lemos, D. R. H., Melo, E. C., Rocha, R. P., Barbosa, L. C. A., & Pinheiro, A. L. (2012). Influence of drying air temperature on the chemical composition of the essential oil of melaleuca. Engenharia na agricultura, 2014: 5-11.

Li, X., Schuler, M. A., & Berenbaum, M. R. (2007). Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annual Review of Entomology, 52, 231-253.

Lima, R. N. (2019). Antitumor and Aedes aegypti Larvicidal Activities of Essential Oils from Piper klotzschianum, P. hispidum, and P. arboreum. Natural Product Communications, 14(7), 1-6.

Maia, J. G. S., Zoghbi, M. G. S., Andrade, E. H. A., Santos, A. S., Silva, M. L., Luz, A. I. R., & Bastos, C. N. (1998). Constituents of the essential oil of Piper aduncum L. growing in the Amazon Region. Flavour and Fragrance Journal, 13(4), 269-272.

NIST – National Institute of Standards and Technology. (1999). Pc version 1.7 of the NIST/EPA/NIH Mass Spectral Library Norwalk. Perkin-Elmer Corp., CT, USA.

Oliveira, G. L., Cardoso, S. K., Celio, R. L. J., Vieira, T. M., Guimarães, E. F., Figueiredo, L. S., Martins, E. R., Moreira, D. L., & Kaplan, M. A. (2012). Chemical study and larvicidal activity against Aedes aegypti of essential oil of Piper aduncum L. (Piperaceae). Anais da Academia Brasileira de Ciências, 52(4), 1227-1234.
Larvicidal activity of essential oils from Piper species against strains of Aedes aegypti (Diptera: Culicidae) resistant to pyrethroids. *Frontiers in Plant Science*, 12, 1-13.

Pohlit, A. M., Pinto, A. C. S., & Mause, R. (2006). *Piper aduncum* L.: Planta pluripotente e Fonte de Substâncias fitoquímicas importantes. *Revista Fitos*, 2(1), 9-18, 2006.

Proksch, P. & Rodríguez, E. (1983). Chromenes and benzofurans of the Asteraceae, their chemistry and biological significance. *Phytochemistry*, 22:2335–2348.

Santana, H. T., Trindade, F. T. T., Stabeli, R. G., Silva, A. A. E., Militão, J. S. L. T., & Fagundo, V. A. (2015). Essential oils of leaves of *Piper* species display larvicidal activity against the dengue vector, *Aedes aegypti* (Diptera: Culicidae). *Revista Brasileira de Plantas Medicinais*, 17(1), 105-111, 2015.

Simas, N. K., Lima, E. C., Conceição, S. R., Kuster, R. M., & Oliveira Folho, A. M. (2004). Produtos naturais para o controle da transmissão da dengue-atividade larvicida de *Myroxylon balsamum* (óleo vermelho) e de terpenóides e fenilpropanóides. *Química Nova*, 27(1), 46-49.

Simões, C. M. O. (2010). *Farmacognosia*: do produto natural ao medicamento. Editora da UFRGS e UFSC, 2010.

Simões, C. M. O., Schenkel, E. P., Mello, J. C. P., Mentz, L. A., & Petrovick, P. R. (2017). *Farmacognosia*: do produto natural ao medicamento. Editora: Artmed.

Souza, P. J. C., Barros, C. A. L., Rocha, J. C. S., Lira, D. S., Monteiro, G. M., & Maia, J. G. S. (2008). Avaliação toxicológica do óleo essencial de *Piper aduncum* L. *Revista Brasileira de Farmacognosia*, 18 (2), p.217-221.

Souza, S. J. O., Ferri, P. H., Fiuza, T. S., Borges, L. L., & Paula, J. R. (2018). Chemical composition and seasonality variability of the *Spiranthera odoratissima* volatile oils leaves. *Revista Brasileira de Farmacognosia*, 28 (1): 16-20.

Taher, M., Amri, M. S., Susanti, D., Kudos, M. B. A., Nor, N. F. A. M., & Syukri, Y. (2020). Medicinal uses, phytochemistry and pharmacological properties of *Piper aduncum* L. *Sains Malysian*, 49(5): 1829-1851.

Torres, L., Rojas, J., Rondón, M., Morales, A., & Nieves, E. (2017). Insecticide activity of *Argentina jahnii* and *Argentina pichinchensis* (Asteraceae) against *Lutzomyia migonei* (Diptera: Psychodidae). *Advanced Biomedical Research*, 6, 1-5.

Valadares, A. C. F., Alves, C. C. F., Alves, J. M., De-Deus, I. P., Oliveira-Filho, J. G., Dos-Santos, T. C., Dias, H. J., Crottì, A. E. M., & Miranda, M. L. D. (2018). Essential oils from *Piper aduncum* inflorescences and leaves: Chemical composition and antifungal activity against *Sclerotinia sclerotiorum*. *Anais da Academia Brasileira de Ciências*, 93(3), 2691-2699.

Vila, R. (2005). Unusual composition of the essential oils from the leaves of *Piper aduncum*. *Flavour and Fragrance Journal*, 20, p.67-69.

WHO – World Health Organization. *Guidelines for laboratory and fields testing of mosquito larvicides*, 2005.