

Essential Oil of Piper Purusianum C.DC (Piperaceae) and Its Main Sesquiterpenes: Biodefensives Against Malaria and Dengue Vectors, Without Lethal Effect on Non-target Aquatic Fauna

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Abstract

The mosquitoes vectors of the genus *Aedes* and *Anopheles* present resistance to several commercial insecticides, which are also toxic to non-predators targets. On the other hand, essential oils are a promising source of insecticides. Thus, in this work, the essential oil from the leaves of *Piper purusatum* was characterized by gas chromatography based approaches and evaluated as biodefensive against malaria and dengue vectors. The main compounds of *P. purusatum* essential oil were β -caryophyllene (57.05%), α -humulene (14.50%) and germacrene D (8.20%). The essential oil inhibited egg hatching (7.6 ± 1.5 to $95.6 \pm 4.5\%$) caused larval death (LC_{50} from 49.84 to 51.60 ppm) and inhibited the action of acetylcholinesterase (IC_{50} of 2.29 μ g/mL), which can be related to the mechanisms of action. On the other hand, the biological activity of β -caryophyllene, α -humulene and germacrene D were higher than the essential oil. In addition, these sesquiterpenes and essential oil did not show a lethal effect on *Toxorhynchites splendens*, *Anisops bouvieri*, *Gambusia affinis* and *Diplonchus indicus* (LC_{50} from 2098.80 to 7707.13 ppm), although *D. indicus* is more sensible (SI/PSF from 48.56 to 252.02 ppm) to essential oil, representing a natural alternative against these relevant vectors.

Introduction

Malaria and dengue are fatal infections diseases caused by etiological agents of the Plasmodium (Plasmodidae) and Flavivirus (Flaviviridae) genera, which in 2019 caused respectively 229 and 390 million infections in all the world (PAHO 2020a; WHO 2021), while in South of America, 65% of these infections occurred in Brazil (WHO 2020; PAHO 2020b). In Brazil, malaria is mainly transmitted by the mosquito of the species *Anopheles darlingi*, while *An. nuneztovari*, *An. triannulatus* and *An. albitarsis* are secondary transmitters (Consoli and Lourenço 1994). These mosquitoes are endemic to the rural area of the State of Amazonas, which concentrates 99% of cases of the disease, with 157.454 infections and 37 deaths reported only 2019 (Ministry of Health 2021a, b). On the other hand, dengue is endemic to urban areas due to the characteristics of the main vector, *Aedes aegypti*, while *Ae. albopictus* is classified as a secondary vector (WHO 2021). In 2019, Brazil notified to the World Health Organization, 1.544.987 infections and 1.077 deaths were caused by dengue (Ministry of Health 2019). Difficulties in controlling dengue and malaria occur because the vectors are resistant to commercial insecticides, which are also toxic to non-target predators (Braga and Valle 2007; Melo-Santos et al. 2010; Nogueira et al. 2010; Aguirre-Obando et al. 2016). This resistance was identified as one determining factor for the dissemination of malaria and dengue epidemics that occurred in Brazil between 2010 and 2016 (Ministry of Health 2021a, b). Actually, plant-based products, especially essential oils, are promising natural insecticides (Qin et al. 2010; Marques et al. 2017; Janarium et al. 2018), for example, essential oils from *Zanthoxylum limonella* Alston (Rutaceae) against *Ae. aegypti* (Soonwera et al. 2017) and *Kaempferia galanga* L. (Zingiberaceae) evaluated against *Ae. vittatus* and *An. maculatus* (Culicidae) (AlSalhi et al. 2020). The *Piper* genus is the largest in the Piperaceae family, with approximately 2.000 plant species (Marques and Kaplan 2015), to which essential oils have shown promising biological activities against mosquitoes of the *Aedes* and *Anopheles* genera (Matasyoh et al. 2011; Villamizar et al. 2017; Kanis et al.

2018). *P. purusanum* C.DC have a restricted distribution in the State of Amazonas, Brazil (Guimarães et al. 2020), with no chemical and biological knowledge reported so far. Thus, in the present work, the essential oil from the leaves of *P. purusanum* was characterized by GC-MS and GC-FID, and evaluated as biodefensive against malaria and dengue vectors based on inhibition egg hatching, larvicidal and anticholinesterase activities, and toxicity against non-target predators. In addition, the main compounds from essential oil were also evaluated as potential biodefensive agents.

Materials And Methods

Instruments and chemicals

A knife mill (SL-31, Solab, Brazil), a heating mantle (52E, Fisatom, Brazil) and a chiller (Julabo GMBH, Biovera, Brazil) were used in the essential oil distillation procedure. A pH meter (PHS3BW, Bel Engineering, Brazil) and a refractometer (Master-T, Atago, Brazil) were used to measure the physical chemistry properties of the essential oil. A GC-MS system constituted by a Trace GC Ultra gas chromatograph coupled to an ISQ mass spectrometer (Thermo Scientific, USA) and a GC-FID system (G-1860 Plus, Agilent equipment, USA) were used for the chemical analysis of the essential oil. A microplate reader (ELx800, Biotek, USA) was used in the acetylcholinesterase (AChE) assay. A microscope (Stemi 200-C, Zeiss, Germany) was used in the egg hatch inhibition test.

The AChE from *Electrophorus electricus* (200-1.000 units/mg protein), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) (98%), acetylcholine iodide (AChI) (97%), tris(hydroxymethyl)aminomethane (99.8%), β -caryophyllene (80%), α -humulene (90%), MeOH (99.8%), temephos (Pestanal® 95%), anhydrous sodium sulfate (Na_2SO_4) (99%), *n*-alkanes series (C8-C30) (Supelco) and dimethylsulfoxide (DMSO) (99.7%) were purchased from Merck, Brazil. The germacreno D (99%) was purchased from Jinlan Pharm-Drugs Technology, Hangzhou, China.

Plant collection and distillation of essential oil

The leaves of *P. purusanum* were collected in September, 2015, in Manaus, Amazonas, Brazil (Latitude 2°46'58.2"S, Longitude 60°05'2.8"W). The voucher (HUAM No. 11425) was deposited in the herbarium at Federal University of Amazonas. For the essential oil extraction, 50 g of the powdered leaves of *P. purusanum* were subjected to hydrodistillation using a Clevenger-type apparatus for 1 h. The essential oil was dried using anhydrous sodium sulfate (Na_2SO_4) and stored in an amber glass vial at -4 °C until analysis (de Oliveira et al. 2020). The values of pH, refractive index (Anjali et al. 2012), density and yield also were determined (Girard et al. 2007). All of the procedures were conducted in triplicate.

GC-MS and GC-FID analyses of essential oil

The GC-MS and GC-FID analyses of essential oil from *P. purusanum* were performed according to with previously reported methodology (de Oliveira et al. 2020). The retention index (RI) was obtained using the *n*-alkanes series (C8-C30) and the Van Den Dool and Kratz equation (Van Den Dool and Kratz, 1963). The

identification of each compound was done by comparison of the obtained retention index with the ones found in the literature (Adams 2017; NIST 2020).

Mosquitoes rearing

The *Ae. aegypti* and *Ae. albopictus* mosquitoes were reared at the Laboratório de Malária e Dengue at INPA under controlled temperature (26 ± 3 °C), relative humidity (85%) and photoperiod (24 h) (Silva et al., 2014). Mosquitoes of the species *An. triannulatus*, *An. darlingi*, *An. nuneztovari* and *An. albitarsis* were collected following the WHO methodology (WHO 1975) at the Ramal Brasileirinho (Latitude $3^{\circ}0'42.9''$ S, Longitude $59^{\circ}52'29.9''$ W), Manaus, Amazonas, Brazil. These mosquitoes were identified using taxonomic keys (Gorham et al. 1973; Faran and Linthicum 1981; Consoli and Lourenço 1994) and reared in the same conditions. The blood meal of female mosquitoes was performed in *Mesocricetus auratus* (Cricetidae) hamsters.

Egg hatch inhibition assay

Egg hatch inhibition was evaluated following the method described by Rajkumar et al. (2011) with some modifications. Briefly, the eggs ($n = 100$) were divided into five groups (20 eggs each), distributed in plastic cups (200 mL) and treated for 3 h with concentrations from 1.95 to 31.25 ppm of the essential oil, β -caryophyllene, germacrene D and α -humulene prepared in 1 mL of DMSO. Each concentration contained five replicates. Subsequently, the eggs were transferred to plastic cups containing 100 mL of distilled water. DMSO and temephos also were evaluated at these concentrations. Eggs were observed under a microscope after 120 h to evaluate the percentage of inhibition at each concentration using the formula: number of unhatched eggs / Total number of eggs exposed x 100.

Larvicidal assay

The larvicidal activity was carried out following the WHO (2005) protocol. The 3rd instar larvae ($n = 100$) were distributed in plastic cups (500 mL) containing 249 mL of distilled water and essential oil concentrations (25 to 125 ppm), β -caryophyllene, germacrene D and α -humulene (10 to 50 ppm) prepared in 1 mL of DMSO. Concentrations were followed by five replicates. DMSO was evaluated at concentrations from 25 to 125 ppm, while temephos was evaluated at concentrations from 0.78 to 12.5 ppm. The percentage of activity at each concentration was calculated after 24 h using the equation (1).

$$\text{Larvicida activity \%} = \frac{\text{number of dead larvae}}{\text{number of larvae used}} \times 100 \quad (1)$$

Toxicity assessment in non-target predators

The toxicity of essential oil, β -caryophyllene, germacrene D and α -humulene was evaluated following the methodology described by Sivagnaname and Kalyanasundaram (2004), with some modifications. Briefly, the species *A. bouvieri* (Hemiptera), *D. indicus* (Heteroptera), *T. splendens* (Culicidae) and *G. affinis* (Poeciliidae) were collected at INPA and identified using a taxonomic key (Hamada et al. 2014). Besides,

these samples were acclimated in a tank glass (90 cm in diameter and 40 cm deep) containing 5 L of potable water for 24 h and distributed in plastic cups containing 499 mL of potable water and concentrations of 250 to 2000 ppm prepared in 1 mL of DMSO. The negative control was evaluated at these concentrations, while temephos was evaluated at concentrations from 0.78 to 12.5 ppm. Mortality and slow movements were monitored for 20 days. The Suitability index (SI) or Predator safety factor (PSF) was calculated for each species of predator using the equation (2) (Deo et al. 1988).

$$SI/PSF = \frac{LC_{50} \text{ of non - target organism}}{LC_{50} \text{ of target vector specie}} \quad (2)$$

Acetylcholinesterase inhibition assay (AChE)

Inhibition of AChE was performed by the colorimetric methodology described by Ellman et al. (1961) with modifications. Briefly, neostigmine (1 mg) and AChE (10 µL) were prepared in phosphate buffer (1 mL) of a 0.1M solution at pH 8, while β -caryophyllene, germacrene D, α -humulene and essential oil (1 mg) were prepared in MeOH (1 mL) and evaluated at concentrations from 0.07 to 10 ppm. The test was performed in triplicate using a 96-well microplate incubated in an environment protected from light for 15 minutes. Negative and positive controls were evaluated under these conditions. Absorbance readings were taken ten times at a wavelength of 405 nm for 30 min with an interval of 5 min. The percentage of inhibition at each concentration was calculated according to the equation (3).

$$\text{Inhibition \%} = A^2 - (A^1 - A^3) \times \frac{100}{A^2} \quad (3)$$

where: A^1 is the absorption of the samples and the enzyme, A^2 is the absorption of the enzyme without the sample, and A^3 is the sample absorption without the enzyme.

Statistical analysis

Probit analysis (Finney 1971) was used to identify the LC_{50} and LC_{90} using the PoloPlus program (Leora Software Berkeley, CA, USA). In the AChE assay, absorbances were normalized and analyzed using the non-linear regression method to identify the IC_{50} . All data were analyzed using ANOVA followed by Tukey test ($p < 0.05$) through the GraphPad Prism statistical program version 7 (de Oliveira et al. 2020).

Results And Discussion

Essential oil analysis

The essential oil was extracted from *P. purusanum* leaves with a yield of $4.2 \pm 0.7\%$, pH of 5.3 ± 1.9 , density of 0.974 g/cm^3 and refractive index of 1.941. The GC-MS and GC-FID analysis pointed sesquiterpenes oxygenated and sesquiterpenes hydrocarbons, especially β -caryophyllene (57.50%), α -humulene (14.50%), germacrene D (8.20%) (Fig. 1), *E*-nerolidol (4.20%) and caryophyllene oxide (3.46%)

as the main compounds in the essential oil (Table 1). This chemical profile is similar to that observed in the essential oils of the species *P. amalago* L., *P. umbellatum* L., *P. dilatatum* LC, Rich, *P. leptorum* Kunth, *P. amplum* Kunth, *P. gaudichaudianum* Kunth and *P. crassinervium* Kunth, which demonstrated significant anti-cancer, anti-inflammatory, antibacterial, repellent, anti-fungal, anti-malarial, anti-leishmanial, and larvicidal activities (Guerrini et al. 2009; Andrade et al. 2011; Kelly et al. 2014; Vaz et al. 2016; Ventorim et al. 2016; Ravi et al. 2006; Raphael et al. 2013; Sperotto et al. 2013; Nararak et al. 2020).

Egg hatch assay

The leaves essential oil of *P. purusanum* from 15.62 to 31.25 ppm showed higher activity in inhibiting the hatching of eggs (Table 2) of *Ae. aegypti*, *Ae. albopictus*, *An. albitarsis*, *An. triannulatus*, *An. darlingi* and *An. nuneztovari* (63.3 ± 5.1 to $98.0 \pm 3.4\%$) than temephos (7.0 ± 2.1 to $57.0 \pm 1.1\%$) ($p < 0.05$), as well as to β -caryophyllene (0.6 ± 0.5 to $54.6 \pm 7.2\%$), α -humulene (1.0 ± 1.0 to $61.3 \pm 5.8\%$) and germacrene D (1.3 ± 1.5 to $54.6 \pm 2.5\%$) ($p < 0.05$) sesquiterpenes. Similar results to the essential oil of *P. purusanum* were observed in the species *Limonia acidissima* L. (Rutaceae), *Sophora alopecuroides* (Fabaceae) and *Coleus aromaticus* (Lamiaceae), whose main compounds were niloticin, sophocarpin, and β -caryophyllene, respectively (Reegan et al. 2014; Baranitharan et al. 2017; Shoukat et al. 2020).

The inhibition of hatching of eggs caused by essential oils and commercial insecticides occurs by inhibiting the synthesis of ecdysone, juvenile hormone, and chitin, which causes the death of the embryo due to malformation (Suman et al. 2013). Furthermore, these promising agents also obstruct the chorion, preventing the exchange between CO₂ and O₂ gases resulting in the accumulation of CO₂ within the eggs causing embryo death from anoxia (de Oliveira et al. 2020; Kala et al. 2019; Mehlhorn et al. 2011).

Larvicidal assay

The essential oil of *P. purusanum* showed high activity against *An. nuneztovari*, *An. triannulatus*, *An. darlingi* and *An. albitarsis* larvae (LC₅₀ from 49.84 to 51.61 ppm) (Table 3), as well as against *Ae. aegypti* and *Ae. albopictus* larvae (LC₅₀ from 42.62 to 53.41 ppm) (Table 4). On the other hand, the β -caryophyllene, α -humulene and germacrene D showed higher activity against *Anopheles* (LC₅₀ from 24.49 to 33.08 ppm) and *Aedes* larvae (LC₅₀ from 28.11 to 35.96 ppm) than essential oil ($p < 0.05$). However, the activity of essential oil and sesquiterpenes were lower ($p < 0.05$) than temephos (LC₅₀ from 3.85 to 5.45 ppm) and *Aedes* larvae (LC₅₀ from 3.21 to 2.75 ppm).

The larvicidal activity of the essential oil of *P. purusanum* is in agreement with previously published data to species *P. capense* LF (LC₅₀ from 34.9 to 85.10 ppm), *P. corcovadensis* (Miq.) (LC₅₀ from 30.52 ppm) and *P. alatipetiolatum* Yunck (LC₅₀ of 33.74 ppm) (Piperaceae), which presented significant activity against *Aedes*, *Anopheles* and *Culex* larvae (Govindarajan et al. 2011; Matasyoh et al. 2011; da Silva et al. 2016; de Oliveira et al. 2020). Furthermore, the larvicidal activity of β -caryophyllene, α -humulene and germacrene D corroborates other larvicidal studies (Ravi et al. 2006; Govindarajan and Benelli 2016a, b). Although caryophyllene oxide, α -terpineol, β -selinene and *E*-nerolidol were not investigated in our study,

they are important larvicidal agents (Cheng et al. 2004; Pavela 2015; Huang et al. 2019), and could contribute through a synergic effect to the improvement of the larvicidal activity.

During the experiments, the larvae showed tremors, slow movements, extrusion of the peritrophic membrane and darkening of the whole body. These changes also observed in other essential oil studies are triggered by the collapse of the central nervous system after inhibition of the enzyme acetylcholinesterase, as well as inhibition of the digestive and hormonal system (Abed et al. 2007; Barreto et al. 2007; Al-Mehmadi and Al-Khalaf 2010).

Toxicity to non-target predators

The results of toxicity against non-targeted predators *A. bouvieri*, *T. splendens*, *G. affinis* and *D. indicus* were presented in table 5 and showed that the LC₅₀ values of the essential oil from *P. purusanum* (LC₅₀ from 2506.56 to 7707.13 ppm), β -caryophyllene (LC₅₀ from 4418.14 to 6335.83 ppm), α -humulene (LC₅₀ from 2386.94 to 6082.02 ppm) and germacrene D (LC₅₀ from 2098.80 to 5429.94 ppm) were lower than temephos (LC₅₀ of 4.85 to 5.81 ppm). In addition, the SI/PSF values against *Anopheles* was from 48.57 to 252.02 (Table 6) and SI/PSF values against *Aedes* was from 46.93 to 216.36 (Table 7), suggesting that the LC₅₀ values of the essential oil, β -caryophyllene, α -humulene and germacrene D are not toxic to these predators (Table 3 and 4). Moreover, the swimming activity of predators was not affected by essential oil and sesquiterpenes during the 20 days.

The species *A. bouvieri*, *T. splendens*, *G. Affinis*, *D. Indicus* and *Poecilia reticulata* (Poeciliidae) are important bioindicators of the toxicity of natural plant products (Govindarajan 2016; Benelli et al. 2018). For example, the extract of *Monofilla atlantia* (Roxb.) A.DC (Rutaceae) (LC₅₀ from 0.14 to 23.36 mg/mL) (Sivagnaname and Kalyanasundaram 2004) and the compound thymol (LC₅₀ from 12.51 to 10.99 mg/mL) were more toxic against *P. reticulata* than 1,8-cineole (LC₅₀ from 3997.07 to 1701.93 mg/mL) (Bullangpoti et al. 2018).

Acetylcholinesterase inhibition assay (AChE)

The AChE inhibitory activity of *P. purusanum* essential oil (IC₅₀ of 2.29 ppm) was higher than β -caryophyllene (IC₅₀ of 5.37 ppm), α -humulene (IC₅₀ of 4.36 ppm) and germacrene D (IC₅₀ of 10.91 ppm) (Table 8). Besides, the AChE inhibitory activity was IC₅₀ of 0.77 ppm for neostigmine. The enzymatic action of the essential oil of *P. purusanum* corroborates with species *P. hispidum* Sw., (IC₅₀ of 0.01 ppm), *P. anonifolium* Kunth, (IC₅₀ of 0.01 ppm), *P. aleyreanum* C.DC (IC₅₀ of 10 ppm) (Kelly et al. 2014) and *P. betle* L. (IC₅₀ of 0.11 to 0.47 ppm) (Karak et al. 2018).

The literature demonstrates that several compounds obtained from plants and essential oils present high AChE inhibitory activity, for example, *Acorus calamus* L. (Acoraceae) (IC₅₀ of 9.48 ppm), *Cyperus rotundus* L. (Cyperaceae) (IC₅₀ of 22.05 ppm), *Azadirachta indica* A. Juss. (Meliaceae) (IC₅₀ of 5.8 ppm)

(Vinutha et al., 2007), 1,8-cineole (IC₅₀ of 670 µM), (+)-α-pinene (IC₅₀ of 630 µM) and (+)-pulegone (IC₅₀ of 890 µM) (Houghton et al. 2006).

Inhibition of AChE results in exhaustive stimulation of neurons due to the accumulation of acetylcholine in the synaptic slit, causing the collapse of the central nervous system, and resulting in exhaustive muscle contraction, involuntary muscle contractions, tremors, later lethargic movements that follow the death of the mosquitoes (Vinutha et al. 2007; Al-Mehmadi and Al-Khalaf 2010). These reactions were also observed with *Anopheles* and *Aedes* larvae, as well as extrusion of the peritrophic membrane and darkening of the larvae during exposure to *P. purusanum* essential oil, β-caryophyllene, α-humulene and germacrene D. Due to the complexity of the chemical composition of essential oils, the mechanisms of action are attributed to the various compounds and their ability to penetrate the insects body, acting on specific enzymes of the central nervous system (AlSalhi et al. 2020), digestive and hormonal (Lee et al. 2002; Barreto et al. 2007). The extrusion of the peritrophic membrane is classified as a defense mechanism for larvae to expel toxic compounds from the intestine (Abed et al. 2007). Darkening is caused by inhibition of the endocrine system, causing overlapping cuticles (Demirci et al. 2017). Thus, AChE inhibition can not be interpreted as the only mode of action (Kelly et al. 2014; AlSalhi et al. 2020).

Conclusion

In the present study, the first description of the chemical profile of the essential oil of *P. purusanum* and its insecticidal activity against vectors mosquitoes of malaria and dengue and non-target predators was reported. The obtained results demonstrate that *P. purusanum* essential oil and its main compounds are promising natural insecticide agents against malaria and dengue vectors without toxicity against non-target predators. The investigation of the effects of essential oils and their main compounds on predators allows us to understand several parameters such as toxicity and selectivity. Thus, these results demonstrate that the essential oil of *P. purusanum*, as well as β-caryophyllene, α-humulene and germacrene D can be considered an effective alternative in the control of malaria and dengue.

Declarations

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Ethical Approval

The collection of plant and mosquitoes were authorized by Sistema de Autorização e Informação em Biodiversidade (SISBIO) (No. 78388-1, No. 74151). The blood meal of female mosquitoes performed in hamsters was authorized by the Ethics Committee on the Use of Animals (No. 058/2018, SEI 01280.001882/2018-21). The study was registered in the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen) (No. ADC693C).

Authors Contributions

André C. de Oliveira, Felipe M. A. da Silva, Sergio M. Nunomura, Rosemary A. Roque, Wanderli P. Tadei and Rita C. S. Nunomura: Conceptualization, Methodology, Investigation, Formal analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing and Supervision. Carlos A. P. Lima and Rejane C. Simões: Conceptualization.

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Competing Interests

The authors declare that they have no competing interests.

Consent to Participate Not applicable

Consent to Publish Not applicable

Availability of data and materials Not applicable

References

- Abed, R.A., Cavaşin, G.M., Silva, H.H.G., Geris, R., Silva, I.G., 2007. Morphohistological alterations in larvae of *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) caused by larvicidal oil-resin activity of the medicinal plant *Copaifera reticulata* Ducke (Leguminosae). Rev. Patol. Trop. 36, 75-86.
<https://doi.org/10.5216/rpt.v36i1.1819>
- Adams, R.P., 2017. Identification of essential oil components by gas chromatography/mass spectrometry, 4th Edt. Allured Publishing Corporation, Carol Stream. 809p.
- Aguirre-Obando, O.A., Pietrobon, A.J., Bona, A.C.D., Navarro-Silva, M.A., 2016. Contrasting patterns of insecticide resistance and knockdown resistance (*kdr*) in *Aedes aegypti* populations from Jacarezinho

(Brazil) after a dengue outbreak. Rev. Bras. Entomol. 60, 94–100.

<https://doi.org/10.1016/j.rbe.2015.11.009>

Al-Mehmadi, R.M., Al-Khalaf, A.A., 2010. Larvicidal and histological effects of *Melia azedarach* extract on *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). J. King Saud. Univ. Sci. 22, 77–85.

<https://doi.org/10.1016/j.jksus.2010.02.004>

AlSalhi, M., Elumalai, K., Devanesan, S., Govindarajan, M., Krishnappa, K., Maggi, F., 2020. The aromatic ginger *Kaempferia galanga* L. (Zingiberaceae) essential oil and its main compounds are effective larvicidal agents against *Aedes vittatus* and *Anopheles maculatus* without toxicity on the non-target aquatic fauna. Ind. Crops. Prod. 158: 11312. <https://doi.org/10.1016/j.indcrop.2020.113012>

Andrade, E.H.A., Alves, C.N., Guimarães, E.F., Carreira, L.M.M., Maia, J.G.S., 2011. Variability in essential oil composition of *Piper dilatatum* L.C Rich. Biochem. System. Ecol. 39, 669–

675. <https://doi.org/10.1016/j.bse.2011.05.021>

Anjali, C.H., Sharma, Y., Mukherjee, A., Chandrasekaran, N., 2012. Neem oil (*Azadirachta indica*) nanoemulsion - a potent larvicidal agent against *Culex quinquefasciatus*. Pest Manag Sci 68:158–163.

<https://doi.org/10.1002/ps.2233>

Baranitharan, M., Dhanasekaran, S., Kovendan, K., Murugan, K., Gokulakrishnan, J., Benelli, G., 2017. *Coleus aromaticus* leaf extract fractions: A source of novel ovicides, larvicides and repellents against *Anopheles*, *Aedes* and *Culex* mosquito vectors? Process. Saf. Environ. Prot. 106: 23-33.

<http://dx.doi.org/10.1016/j.psep.2016.12.003>

Barreto, C.F., Cavasin, G.M., Garcia da Silva, H.H., Da Silva, I.G., 2007. Study of morphohistological alterations in larvae of *Aedes aegypti* (Diptera: Culicidae) submitted to the crude ethanol extract *Desapindus saponaria* lin (Sapindaceae). Rev. Patol. Trop. 35, 37–57.

<https://doi.org/10.5216/rpt.v35i1.1891>

Benelli, G., Rajeswary, M., Govindarajan, M., 2016. Towards green oviposition deterrents? Effectiveness of *Syzygium lanceolatum* (Myrtaceae) essential oil against six mosquito vectors and impact on four aquatic biological control agents. Environ. Sci. Pollut. Res. (2018) 25:10218–10227

Braga, I.A., Valle, D., 2007. *Aedes aegypti*: insecticides, mechanisms of action and resistance. Epidemiol. Serv. Saud. 16, 279–293. <https://doi.org/10.5123/S1679-49742007000400006>

Brazilian Ministry of Health, 2021b. Epidemiological Bulletin. [boletim_especial_malaria_1dez20_final.pdf](https://www.gov.br/boletim_especial_malaria_1dez20_final.pdf) (www.gov.br) (Accessed August 12, 2021).

Bullangpoti, V., Mujcharyakul, W., Laksanavilat, N., 2018. Acute toxicity of essential oil compounds (thymol and 1,8-cineole) to insectivorous guppy, *Poecilia reticulata* Peters, 1859. Agric. Nat. Resour. 5: 190-194. <https://doi.org/10.1016/j.anres.2018.06.011>

- Cheng, S.S., Liu, J.Y., Tsai, K.H., Chen, W.J., Chang, S.T., 2004. Chemical composition and mosquito larvicidal activity of essential oils from leaves of different *Cinnamomum osmophloeum* provenances. *J. Agric. Food Chem.* 52, 4395–4400. <https://doi.org/10.1021/jf0497152>
- Consoli, R.A.G.B. and Oliveira, R.L. De., 1994. Main mosquitoes of sanitary importance in Brazil. Primeira ed. Fiocruz, Rio de Janeiro.
- da Silva, M.F.R., Bezerra-Silva, P.C., de Lira, C.S., de Lima Albuquerque, B.N., Agra Neto, A.C., Pontual, E.V., Maciel, J.R., Paiva, P.M.G., Navarro, D.M. do A.F., 2016. Composition and biological activities of the essential oil of *Piper corcovadensis* (Miq). C.DC (Piperaceae). *Exp. Parasitol.* 165. 64-70. [10.1016/j.exppara.2016.03.017](https://doi.org/10.1016/j.exppara.2016.03.017)
- de Oliveira, A.C., Sá, I.S.C., Mesquita, R.S., Pereira, B.L., Pocrifka, L.A., de Souza, T.P., Rodriguez Amado, J.R., Azevedo, S.G., Sanches, E.A., Nunomura, S.M., Roque, R.A., Tadei, W.P., Nunomura, R.C.S., 2020. Nanoemulsion loaded with volatile oil from *Piper alatifoliatum* as an alternative agent in the control of *Aedes aegypti*. *Rev. Bras. Farmacogn.* 30, 667-677. <https://doi.org/10.1007/s43450-020-00092-8>
- Demirci, B., Yusufoglu, H.S., Tabanca, N., Temel, H.E., Bernier, U.R., Agramonte, N.M., Alqasoumi, S.I., Al-Rehaily, A.J., Başer, K.H.C., Demirci, F., 2017. *Rhanterium epapposum* Oliv. essential oil: Chemical composition and antimicrobial, insect-repellent and anticholinesterase activities. *Saudi Pharm. J.* 25, 703–708. <https://doi.org/10.1016/j.jsps.2016.10.009>
- Deo, P.G., Hasan, S.B., Majumdar, S.K., 1988. Toxicity and suitability of some insecticides for household use. *Int. Pest Control.* 30, 118–129
- Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new and rapid colourimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* 7, 88–95. [https://doi.org/10.1016/0006-2952\(61\)90145-9](https://doi.org/10.1016/0006-2952(61)90145-9)
- Faran, M.E. Linthicum, K.J., 1981. A handbook of the Amazon species of *Anopheles Nyssorhynchus* (Diptera: Culicidae). *Mosquito System.* 13, 1-81
- Finney, D.J., 1971. Probit Analysis. Cambridge University Press, London, pp. 68–72
- Forgiarini, A., Esquena, J., González, C., Solans, C., 2001. Formation of nano-emulsions by low-energy emulsification methods at constant temperature. *Langmuir* 17, 2076–2083. <https://doi.org/10.1021/la001362n>
- Girard, E.A., Koehler, H.S., Netto, S.P., 2007. “Craveiro” (*Pimenta Pseudocaryophyllus* (Gomes) *Landrum*). Essential oil, volume, biomass and yield. *Rev Acad* 5:147–165.
- Govindarajan, M., 2011. Larvicidal and repellent properties of some essential oils against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera: Culicidae). *Asian Pac. J. Trop Med.* 4, 106–111. [https://doi.org/10.1016/S1995-7645\(11\)60047-3](https://doi.org/10.1016/S1995-7645(11)60047-3)

- Govindarajan, M., Benelli, G., 2016a. Eco-friendly larvicides from Indian plants: Effectiveness of lavandulyl acetate and bicyclogermacrene on malaria, dengue and Japanese encephalitis mosquito vectors. *Ecotoxicol. Environ. Saf.* 133, 395–402. <https://doi.org/10.1016/j.ecoenv.2016.07.035>
- Govindarajan, M., Benelli, G., 2016b. α -Humulene and β -elemene from *Syzygium zeylanicum* (Myrtaceae) essential oil: highly effective and eco-friendly larvicides against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus* (Diptera: Culicidae). *Parasitol. Res.* 115, 2771–2778. <https://doi.org/10.1007/s00436-016-5025-2>
- Govindarajan, M., Mathivanan, T., Elumalai, K., Krishnappa, K., Anandan, A., 2011. Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *Asian Pac. J. Trop. Biomed.* 1, 43–48. [https://doi.org/10.1016/S2221-1691\(11\)60066-X](https://doi.org/10.1016/S2221-1691(11)60066-X)
- Guerrini, A., Sacchetti, G., Rossi, D., Paganetto, G., Muzzoli, M., Andreotti, E., Tognolini, M., Maldonado, M.E., Bruni, R., 2009. Bioactivities of *Piper aduncum* L. and *Piper obliquum* Ruiz & Pavon (Piperaceae) essential oils from Eastern Ecuador. *Environ. Toxicol. Pharmacol.* 27, 39–48. <https://doi.org/10.1016/j.etap.2008.08.002>
- Guimarães, E.F.; Queiroz, G.A.; Medeiros, E.V.S.S., 2020. *Piper* in Flora do Brazil. Botanical Garden of Rio de Janeiro. Available at: <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB86652> (Accessed 9 January 2021).
- Hamada, N., Nessimian, J.L., Querino, R.B., 2014. Aquatic insects in the Brazilian Amazon: taxonomy, biology and ecology. Publisher INPA. 724p.
- Houghton, P., Ren, Y., Howes, M.J., 2006. Acetylcholinesterase inhibitors from plants and fungi. *Nat. Prod. Rep.* 23: 181-199. <https://doi.org/10.1039/b508966m>
- Huang, Y., Lin, M., Jia, M., Hu, J., Zhu, L., 2019. Chemical composition and larvicidal activity against *Aedes* mosquitoes of essential oils from *Arisaema fargesii*. *Pest Manag. Sci.* 76: 534-542. <https://doi.org/10.1002/ps.554>
- Janaki, S., Zandi-Sohani, N., Ramezani, L., Szumny, A., 2018. Chemical composition and insecticidal efficacy of *Cyperus rotundus* essential oil against three stored product pests. *Int. Biodeter. Biodegradation.* 133, 93–98. <https://doi.org/10.1016/j.ibiod.2018.06.008>
- Kala, S., Naik, S.N., Patanjali, P.K., Sogan, N., 2019. Neem oil water-dispersible tablet as effective larvicide, ovicide and oviposition deterrent against *Anopheles culicifacies*. *South African J. Bot.* 123, 387–392. <https://doi.org/10.1016/j.sajb.2019.03.033>
- Kanis, L.A., Rabelo, B.D., Moterle, D., Custódio, K.M., de Oliveira, J.G., de Lemos, A.B., da Silva, O.S., Zepon, K.M., Magnago, R.F., Prophiro, J.S., 2018. *Piper ovatum* (Piperaceae) extract/starch-cellulose films to

control *Aedes aegypti* (Diptera: Culicidae) larvae. Ind. **Crops**. Prod. 122, 148–155.

<https://doi.org/10.1016/j.indcrop.2018.05.055>

Karak, S., Acharya, J., Begum, S., Mazumdar, I., Kundu, R., De, B., 2018. Essential oil of *Piper betle* L. leaves: Chemical composition, anti-acetylcholinesterase, anti- β -glucuronidase and cytotoxic properties. J. Appl. Res. Medic. Aromat. Plant. 10, 85–92. <https://doi.org/10.1016/j.jarmap.2018.06.006>

Kelly, J., Silva, R., Pinto, L.C., Burbano, R.M.R., Montenegro, R.C., Guimarães, E.F., Helena, E., Andrade, A., Guilherme, J., Maia, S., 2014. Essential oils of Amazon *Piper* species and their cytotoxic, antifungal, antioxidant and anti-cholinesterase activities. Ind. **Crops**. Prod. 58, 55–60.

<https://doi.org/10.1016/j.indcrop.2014.04.006>

Lee, S., Peterson, C.J., Coats, J.R., 2002. Fumigation toxicity of monoterpenoids to several stored product insects. J. Stored Prod. Res. 39, 77–85. [https://doi.org/10.1016/S0022-474X\(02\)00020-6](https://doi.org/10.1016/S0022-474X(02)00020-6)

Marques, A.M., Kaplan, M.A.C., 2015. Active metabolites of the genus *Piper* against *Aedes aegypti*: Natural alternative sources for dengue vector control. Univ. Sci. 20, 61–82.

<https://doi.org/10.11144/Javeriana.SC20-1.amgp>

Marques, A.M., Velozo, L.S., Carvalho, M.A., Serdeiro, M.T., Honório, N.A., Kaplan, M.A.C., Maleck, M., 2017. Larvicidal activity of *Ottonia anisum* metabolites against *Aedes aegypti*: A potential natural alternative source for mosquito vector control in Brazil. J. Vector Borne Dis. 54, 61–68.

Matasyoh, J.C., Wathuta, E.M., Kariuki, S.T., Chepkorir, R., 2011. Chemical composition and larvicidal activity of *Piper capense* essential oil against the malaria vector, *Anopheles gambiae*. J. Asia Pac. Entomol. 14, 26–28. <https://doi.org/10.1016/j.aspen.2010.11.005>

Mehlhorn, H., Abdel-ghaffar, F., Semmler, M., 2011. Ovicidal effects of a neem seed extract preparation on eggs of body and head lice 1299–1302. <https://doi.org/10.1007/s00436-011-2374-8>

Melo-Santos, M.A.V., Varjal-Melo, J.J.M., Araújo, A.P., Gomes, T.C.S., Paiva, M.H.S., Regis, L.N., Furtado, A.F., Magalhaes, T., Macoris, M.L.G., Andrighetti, M.T.M., Ayres, C.F.J., 2010. Resistance to the organophosphate temephos: Mechanisms, evolution and reversion in an *Aedes aegypti* laboratory strain from Brazil. Acta Trop. 113, 180–189. <https://doi.org/10.1016/j.actatropica.2009.10.015>

Ministry of Health of Brazil, 2021a. Malária. **Malária – Português (Brasil) (www.gov.br)** (Accessed August 12, 2021).

Nararak, J., Sathantriphop, S., Kongmee, M., Mahiou-Leddet, V., Ollivier, E., Manguin, S., Chareonviriyaphap, T., 2019. Excito-repellent activity of β -caryophyllene oxide against *Aedes aegypti* and *Anopheles minimus*. Acta Trop. 197, 105030.

<https://doi.org/10.1016/j.actatropica.2019.05.021>

National Institute of Standards and Technology (NIST), 2011. Standard reference database. NIST Chemistry WebBook. <http://webbook.nist.gov/chemistry> (Accessed November 24, 2020).

Nogueira, A.J.A., Domingues, I., Agra, A.R., Monaghan, K., Amadeu, M.V.M., 2010. Critical Review: Cholinesterase and glutathione-S-transferase activities in freshwater. *Environ. Toxicol. Chem.* 9, 5–18. <https://doi.org/10.1002/etc.23>.

Pan American Health Organization (PAHO), 2020a. WHO calls for reinvigorated action to fight malaria. [OMS pede ação revigorada para combater a malária - OPAS/OMS | Organização Pan-Americana da Saúde \(paho.org\)](#) (Accessed August 12, 2021).

Pan American Health Organization (PAHO), 2020b. Dengue cases in the Americas reach 1.6 million during the pandemic. [Casos de dengue nas Américas chegam a 1,6 milhão, o que destaca a necessidade do controle de mosquitos durante a pandemia - OPAS/OMS | Organização Pan-Americana da Saúde \(paho.org\)](#) (Accessed August 12, 2021).

Pavela, R., 2015. Acute toxicity and synergistic and antagonistic effects of the aromatic compounds of some essential oils against *Culex quinquefasciatus* Say larvae. *Parasitol. Res.* 114, 3835–3853. <https://doi.org/10.1007/s00436-015-4614-9>

Qin, W., Huang, S., Li, C., Chen, S., Peng, Z., 2010. Biological activity of the essential oil from the leaves of *Piper sarmentosum* Roxb. (Piperaceae) and its chemical constituents on *Brontispa longissima* (Gestro) (Coleoptera: Hispididae). *Pestic. Biochem. Physiol.* 96, 132–139. <https://doi.org/10.1016/j.pestbp.2009.10.006>

Rajkumar, S., Jebanesan, A., Nagarajan, R., 2011. Effect of leaf essential oil of *Coccinia indica* on egg hatchability and different larval instars of malarial mosquito *Anopheles stephensi*. *Asian Pac. J. Trop. Med.* 4, 948–951. [https://doi.org/10.1016/S1995-7645\(11\)60224-1](https://doi.org/10.1016/S1995-7645(11)60224-1)

Raphael, V., Borges, D.A., Ribeiro, A.F., Anselmo, C.D.S., Cabral, L.M., Sousa, V.P. De., 2013. Development of a high-performance liquid chromatography method for quantification of isomers β -caryophyllene and α -humulene in copaiba oleoresin using the Box-Behnken design. *J. Chromatogr. B.* 940, 35–41. <https://doi.org/10.1016/j.jchromb.2013.09.024>

Ravi Kiran, S., Bhavani, K., Sita Devi, P., Rajeswara Rao, B.R., Janardhan Reddy, K., 2006. Composition and larvicidal activity of leaves and stem essential oils of *Chloroxylon swietenia* DC against *Aedes aegypti* and *Anopheles stephensi*. *Bioresour. Technol.* 97, 2481–2484. <https://doi.org/10.1016/j.biortech.2005.10.003>

Reegan, A.D., Gandhi, M.R., Paulraj, M.G., Balakrishna, K., Ignacimuthu, S., 2014. Effect of niloticin, a protolimonoid isolated from *Limonia acidissima* L. (Rutaceae) on the immature stages of dengue vector *Aedes aegypti* L. (Diptera: Culicidae). *Acta Trop.* 139, 67–76. <https://doi.org/10.1016/j.actatropica.2014.07.002>

- Shoukat, R.F., Shakeel, M., Rizvi, S.A.H., Zafar, J., Zhang, Y., Freed, S., Xu, X., Jin, F., 2020. Larvicidal, ovicidal, synergistic, and repellent activities of *Sophora alopecuroides* and its dominant constituents against *Aedes albopictus*. *Insects* 11, 1-13. <https://doi.org/10.3390/insects11040246>
- Silva, A.P., Alves, W.S., Martins, A., Tadei, W.P, Santos, J.M.M, 2014. Adaptation of a simplified bioassay to assess susceptibility status in larvae of *Anopheles darlingi* and *Anopheles marajoara* to pyrethroid deltamethrin. *Brazilian Entomol. Soc.* 9, 1-8. doi: [10.14295/BA.v9.139](https://doi.org/10.14295/BA.v9.139)
- Sivagnaname, N., Kalyanasundaram, M., 2004. Laboratory evaluation of methanolic extract of *Atlantia monophylla* (Family: Rutaceae) against immature stages of mosquitoes and non-target organisms, *Mem. Inst. Oswaldo Cruz.* 99, 115–118. doi: [10.1590/s0074-0276200400010002](https://doi.org/10.1590/s0074-0276200400010002)
- Soonwera, M., Phasomkusolsi, S., 2017. Adulticidal, larvicidal, pupicidal and oviposition deterrent activities of essential oil from *Zanthoxylum limonella* Alston (Rutaceae) against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say). *Asian Pac. J. Trop. Biomed.* 7: 967-978. <https://doi.org/10.1016/j.apjtb.2017.09.019>.
- Sperotto, A.R.M., Moura, D.J., Péres, V.F., Damasceno, F.C., Caramão, E.B., Henriques,
- Suman, D.S., Wang, Y., Bilgrami, A.L., Gaugler, R., 2013. Activity of three insect growth regulators against *Aedes* and *Culex* mosquitoes. *Acta Trop.* 128, 103–109. <https://doi.org/10.1016/j.actatropica.2013.06.025>
- Van Den Dool H, Kratz P. D., 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogra A.* 11, 463–471. [https://doi.org/10.1016/S0021-9673\(01\)80947-X](https://doi.org/10.1016/S0021-9673(01)80947-X)
- Vaz, C., Torres, R.B., Bernacci, L.C., Guimarães, E.F., Haber, L.L., Facanali, R., Vieira, M.A.R., Quecini, V., Ortiz, M., Marques, M., 2016. The chemical composition and antibacterial activity of eleven *Piper* species from distinct rainforest areas in Southeastern Brazil. *Ind. Crops. Prod.* 94, 528–539. <https://doi.org/10.1016/j.indcrop.2016.09.028>
- Ventorim, F., Paula, R. De, Caldeira, I., Alvarenga, A., Kelly, S., Bertolucci, V., Alves, A., Alvarenga, D., Eduardo, J., Pereira, B., 2016. Essential oil of monkey-pepper (*Piper aduncum* L.) cultivated under different light environments. *Ind. Crops. Prod.* 85, 251–257. <https://doi.org/10.1016/j.indcrop.2016.03.016>
- Villamizar, L.H., Cardoso, M. das G., de Andrade, J., Teixeira, M.L., Soares, M.J., 2017. Linalool, a *Piper aduncum* essential oil component, has selective activity against *Trypanosoma cruzi* trypomastigote forms at 4° C. *Mem. Inst. Oswaldo Cruz.* 112, 131–139. <https://doi.org/10.1590/0074-02760160361>
- Vinutha, B., Prashanth, D., Salma, K., Sreeja, S.L., Pratiti, D., Padmaja, R., Radhika, S., Amit, A., Venkateshwarlu, K., Deepak, M., 2007. Screening of selected Indian medicinal plants for

acetylcholinesterase inhibitory activity. J. Ethnopharmacol. 109, 359–363.

<https://doi.org/10.1016/j.jep.2006.06.014>

World Health Organization (WHO), 1975. Manual on practical entomology in malaria. Part II. Methods and Techniques. Geneva, World Health Organization (WHO Offset Publication 13).

World Health Organization (WHO), 2005. Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization Commun. Dis. Control. Prev. Erad. Who Pestic. Eval. Scheme 1–41. Ref: WHO/CDS/WHOPES/GCDPP/2005.11

World Health Organization (WHO), 2020. World malaria report 2020: 20 years of global progress & challenges. Geneva: World Health Organization; 2020. Licence: CC BY-NC-AS 3.0 IGO.

World Health Organization (WHO), 2021. Dengue e dengue grave. [Dengue e dengue grave \(who.int\)](https://www.who.int) (Accessed august 12, 2021).

Tables

Table 1. Chemical composition of essential oil of leaves from *Piper purusum*.

Compounds	Area (%) ^a	RI ^{calc}	RI ^{lit}	Identification
Linalool	0.52	1095	1096	RI, MS
α -Terpineol	1.54	1186	1188	RI, MS
β -caryophyllene	57.05	1419	1417	RI, MS
α -Humulene	14.50	1454	1452	RI, MS
γ -Muurolene	0.60	1479	1478	RI, MS
Germacrene D	8.20	1481	1484	RI, MS
β -Selinene	2.10	1490	1489	RI, MS
α -Selinene	4.42	1498	1500	RI, MS
δ -Cadinene	2.40	1523	1525	RI, MS
<i>E</i> -Nerolidol	4.20	1563	1561	RI, MS
Caryophyllene oxide	3.46	1583	1552	RI, MS
Eudesmol	0.84	1662	1663	RI, MS
Total of identified (%)	94			

RI^{calc} - Retention index calculated using n-alkanes (C8-C30) on the TR5 column. RI^{lit} - Retention index literature (Adams 2007; NIST 2020; in-house library). ^a - Relative area calculated from the peak area

relative to the total peak area in the GC-FID chromatogram. MS - Mass spectra compared with literature (Adams 2007; NIST 2020; in-house library). Mach in percentage.

Table 2. Inhibitory activity from Piper purusatum essential oil and its main sesquiterpenes in eggs of Aedes and Anopheles eggs.

		Concentration (ppm)				
		1.95	3.90	7.81	15.62	31.25
Essential oil	<i>Ae. aegypti</i>	0.0 ± 0.0 ^a	7.6 ± 1.5 ^a	20.0 ± 2.0 ^a	63.3 ± 5.1 ^a	84.6 ± 3.0 ^a
	<i>Ae. albopictus</i>	0.0 ± 0.0 ^a	12.3 ± 1.5 ^b	45.6 ± 3.0 ^b	72.0 ± 3.0 ^{bf}	81.6 ± 2.5 ^b
	<i>An. albitarsis</i>	0.0 ± 0.0 ^a	16.6 ± 1.5 ^c	37.3 ± 2.5 ^c	88.3 ± 2.5 ^c	95.6 ± 4.5 ^c
	<i>An. triannulatus</i>	0.0 ± 0.0 ^a	12.0 ± 2.0 ^{db}	54.3 ± 3.0 ^d	90.0 ± 3.6 ^d	94.6 ± 5.5 ^{dc}
	<i>An. darlingi</i>	0.0 ± 0.0 ^a	17.0 ± 2.0 ^{ec}	49.3 ± 2.5 ^e	96.0 ± 4.0 ^e	98.0 ± 3.4 ^e
	<i>An. nuneztovari</i>	0.0 ± 0.0 ^a	11.3 ± 2.0 ^{fb}	42.6 ± 3.0 ^f	73.0 ± 3.0 ^f	87.3 ± 2.5 ^f
β -caryophyllene	<i>Ae. aegypti</i>	0.0 ± 0.0 ^a	2.6 ± 1.5 ^a	10.3 ± 2.5 ^a	25.6 ± 4.1 ^a	45.6 ± 3.0 ^a
	<i>Ae. albopictus</i>	0.0 ± 0.0 ^a	1.6 ± 1.5 ^b	9.3 ± 1.5 ^b	34.3 ± 3.0 ^b	50.6 ± 1.5 ^b
	<i>An. albitarsis</i>	0.0 ± 0.0 ^a	2.0 ± 0.1 ^{ab}	10.3 ± 1.5 ^a	27.6 ± 3.2 ^c	48.3 ± 3.0 ^c
	<i>An. triannulatus</i>	0.0 ± 0.0 ^a	1.3 ± 1.5 ^{cbd}	7.0 ± 2.0 ^c	34.3 ± 2.5 ^{db}	47.3 ± 3.2 ^d
	<i>An. darlingi</i>	0.0 ± 0.0 ^a	0.6 ± 0.5 ^{de}	9.3 ± 1.5 ^{db}	33.3 ± 2.5 ^e	54.6 ± 7.2 ^e
	<i>An. nuneztovari</i>	0.0 ± 0.0 ^a	1.3 ± 1.5 ^{eb}	10.6 ± 0.2 ^a	36.0 ± 2.6 ^f	48.6 ± 1.5 ^{fc}
α -Humulene	<i>Ae. aegypti</i>	0.0 ± 0.0 ^a	2.3 ± 2.0 ^a	15.6 ± 1.5 ^a	35.6 ± 1.5 ^a	58.3 ± 1.2 ^a
	<i>Ae. albopictus</i>	0.0 ± 0.0 ^a	3.6 ± 2.5 ^b	19.3 ± 1.5 ^b	39.3 ± 1.5 ^b	57.0 ± 5.2 ^b
	<i>An. albitarsis</i>	0.0 ± 0.0 ^a	5.3 ± 3.2 ^c	16.6 ± 0.5 ^c	41.3 ± 1.4 ^c	55.0 ± 3.6 ^c
	<i>An.</i>	0.0 ±		19.3 ±	35.6 ± 1.2 ^a	57.6 ±

	<i>triannulatus</i>	0.0 ^a	1.0 ± 1.0 ^d	1.5 ^{db}		3.5 ^{ab}
	<i>An. darlingi</i>	0.0 ± 0.0 ^a	5.0 ± 2.0 ^{ec}	18.0 ± 2.6 ^e	43.3 ± 1.8 ^d	61.3 ± 5.8 ^d
	<i>An. nuneztovari</i>	0.0 ± 0.0 ^a	4.3 ± 1.5 ^{fbe}	18.3 ± 1.5 ^{fe}	36.3 ± 2.0 ^a	51.0 ± 3.6 ^e
Germacrene D	<i>Ae. aegypti</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	5.6 ± 1.4 ^a	24.3 ± 3.0 ^a	51.6 ± 3.0 ^a
	<i>Ae. albopictus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	1.3 ± 1.5 ^b	25.6 ± 1.4 ^b	49.0 ± 2.0 ^b
	<i>An. albitarsis</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	4.3 ± 1.5 ^c	20.6 ± 1.5 ^c	48.0 ± 3.0 ^c
	<i>An. triannulatus</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	5.6 ± 2.0 ^a	18.3 ± 1.4 ^d	49.6 ± 2.5 ^d
	<i>An. darlingi</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	1.3 ± 1.5 ^{db}	16.0 ± 2.6 ^e	51.3 ± 2.0 ^a
	<i>An. nuneztovari</i>	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	3.6 ± 1.3 ^f	22.3 ± 1.5 ^f	54.6 ± 2.5 ^f
Temephos	<i>Ae. aegypti</i>	0.0 ± 0.0 ^a	11.0 ± 2 ^a	20.9 ± 1.0 ^a	39.0 ± 2.2 ^a	49.1 ± 2.4 ^a
	<i>Ae. albopictus</i>	0.0 ± 0.0 ^a	9.2 ± 1.1 ^b	15.1 ± 2.2 ^b	32.3 ± 3.2 ^b	49.6 ± 1.5 ^a
	<i>An. albitarsis</i>	0.0 ± 0.0 ^a	11.5 ± 2.4 ^a	17.0 ± 3.0 ^c	44.0 ± 2.4 ^c	56.0 ± 2.6 ^b
	<i>An. triannulatus</i>	0.0 ± 0.0 ^a	13.0 ± 2.2 ^c	19.0 ± 3.0 ^d	34.1 ± 2.1 ^d	57.0 ± 1.1 ^c
	<i>An. darlingi</i>	0.0 ± 0.0 ^a	7.0 ± 2.1 ^d	15.1 ± 1.1 ^{eb}	37.0 ± 3.2 ^e	46.0 ± 1.1 ^d
	<i>An. nuneztovari</i>	0.0 ± 0.0 ^a	12.5 ± 3.0 ^{ec}	19.0 ± 3.0 ^{fd}	34.0 ± 3.2 ^{fd}	44.0 ± 1.7 ^e

Each value represents the mean of five replicates ± SD. Values carrying different letters in each column are statistical differences by ANOVA Tukey's test $p < 0.05$. The negative control (1.95 to 31.25 ppm) presented eggs hatched from 97.5 ± 2.1 to 100 ± 0.0%.

Table 3. Larvicidal activity of essential oil from *Piper purusanum* and its main sesquiterpenes in *Anopheles* larvae.

	Larvae	LC ₅₀ (ppm) (LCL-UCL)	LC ₉₀ (ppm) (LCL-UCL)	χ^2 (Df)	Slope \pm SE
Essential oil	<i>An. nuneztovari</i>	51.60 ^a (40.769-61.806)	101.17 (82.016-146.279)	7.1(3)	4.383 \pm 0.331
	<i>An. triannulatus</i>	51.61 ^a (43.164-59.634)	96.07 (81.187-124.824)	5.0(3)	4.750 \pm 0.353
	<i>An. darlingi</i>	49.84 ^a (42.553-56.776)	91.82 (78.902-114.696)	4.2(3)	4.830 \pm 0.357
	<i>An. albitarsis</i>	50.05 ^a (38.915-60.469)	100.91 (81.039-149.314)	7.2(3)	4.209 \pm 0.321
β -caryophyllene	<i>An. nuneztovari</i>	28.86 ^a (24.548-33.350)	51.82 (42.994-3.001)	5.9(3)	5.041 \pm 0.404
	<i>An. triannulatus</i>	26.52 ^a (24.831-28.197)	46.51 (42.770-51.738)	0.6(3)	5.253 \pm 0.412
	<i>An. darlingi</i>	25.14 ^a (23.172-27.158)	54.73 (48.603-63.868)	2.8(3)	3.794 \pm 0.308
	<i>An. albitarsis</i>	26.36 ^a (24.437-28.336)	53.92 (48.308-62.182)	0.6(3)	4.124 \pm 0.331
α -Humulene	<i>An. nuneztovari</i>	26.63 ^a (24.832-28.423)	49.56 (45.135-55.885)	0.3(3)	4.749 \pm 0.378
	<i>An. triannulatus</i>	33.08 ^b (31.010-35.315)	61.41 (54.978-71.371)	0.2(3)	4.769 \pm 0.424
	<i>An. darlingi</i>	30.36 ^{ab} (28.020-32.936)	68.88 (59.600-83.958)	0.4(3)	3.602 \pm 0.323
	<i>An. albitarsis</i>	37.42 ^c (34.558-40.982)	82.58 (69.875-104.840)	2.0(3)	3.729 \pm 0.365
Germacrene D	<i>An. nuneztovari</i>	32.36 ^a (30.378-34.459)	58.68 (52.910-67.430)	0.4(3)	4.958 \pm 0.432
	<i>An. triannulatus</i>	30.31 ^a (28.294-32.424)	58.53 (52.427-67.784)	0.3(3)	4.485 \pm 0.385
	<i>An. darlingi</i>	24.49 ^b (22.803-26.165)	45.11 (41.294-50.407)	0.1(3)	4.833 \pm 0.372

	<i>An. albitarsis</i>	31.22 ^a (29.333-33.178)	55.46 (50.374-63.004)	0.4(3)	5.136 ± 0.437
Temephos	<i>An. nuneztovari</i>	3.93 ^a (3.401-4.509)	14.29 (11.810-18.178)	0.2(3)	2.287 ± 0.174
	<i>An. triannulatus</i>	3.85 ^a (3.371-4.369)	11.95 (10.085-14.785)	0.1(3)	2.606 ± 0.196
	<i>An. darlingi</i>	5.21 ^b (4.644-5.828)	13.71 (11.750-16.676)	0.2(3)	3.050 ± 0.234
	<i>An. albitarsis</i>	5.45 ^b (4.787-6.182)	17.72 (14.736-22.382)	0.4(3)	2.503 ± 0.189

LC₅₀ and LC₉₀ - Concentrations needed to kill 50% and 90% of larvae. LCL - Lower confidence limit of 95%. UCL - Upper confidence limit of 95%. $\chi^2 - p < 0.05$. level of significance of *Chi*-square value. Df - Degree of freedom. Letters in the same column indicate statistical differences (ANOVA and Tukey $p < 0.05$). No mortality was observed in the negative control.

Table 4. Larvicidal activity of essential oil from *Piper purusanum* and its main sesquiterpenes in *Aedes* larvae.

	Larvae	LC ₅₀ (ppm) (LCL-UCL)	LC ₉₀ (ppm) (LCL-UCL)	χ^2 (Df)	Slope \pm SE
Essential oil	<i>Ae. aegypti</i>	53.41 ^b (49.773-56.940)	91.01 (84.285-99.953)	2.8(3)	5.536 \pm 0.414
	<i>Ae. albopictus</i>	42.62 ^a (34.627-49.929)	88.63 (74.059-116.525)	4.3(3)	4.030 \pm 0.315
β -caryophyllene	<i>Ae. aegypti</i>	29.97 ^a (28.313-31.611)	48.34 (44.802-53.356)	0.2(3)	6.170 ⁺ -0.512
	<i>Ae. albopictus</i>	31.09 ^b (29.218-33.021)	54.92 (49.942 to 62.313)	0.7(3)	5.187 ⁺ -0.447
α -Humulene	<i>Ae. aegypti</i>	28.11 ^a (26.301-29.940)	51.100 (46.568-57.626)	0.1(3)	4.940 \pm 0.403
	<i>Ae. albopictus</i>	28.89 ^b (27.199-30.570)	48.28 (44.556-53.554)	0.5(3)	5.748 \pm 0.474
Germacrene D	<i>Ae. aegypti</i>	35.96 ^b (33.953-38.183)	61.46 (55.474-70.817)	0.1(3)	5.507 \pm 0.513
	<i>Ae. albopictus</i>	33.51 ^a (31.270-36.014)	66.43 (58.534-79.065)	0.0(3)	4.313 \pm 0.393
Temephos	<i>Ae. aegypti</i>	3.21 ^b (2.936-3.529)	6.63 (5.833-7.797)	2.8(3)	4.082 \pm 0.312
	<i>Ae. albopictus</i>	2.75 ^a (2.210-3.440)	5.25 (4.069-8.442)	6.4(3)	4.560 \pm 0.368

LC₅₀ and ₉₀ - Concentrations needed to kill 50% and 90% of larvae. LCL - Lower confidence limit of 95%. UCL - Upper confidence limit of 95%. χ^2 - Non significant Chi-square ($p < 0.05$). Df - Degree of freedom. Letters in the same column indicate statistical differences (ANOVA and Tukey $p < 0.05$). No mortality was observed in the negative control.

Table 5. Toxicity of essential oil from *Piper purus* and its main sesquiterpenes in non-target predators.

	Predators	LC ₅₀ (ppm) (LCL-UCL)	LC ₉₀ (ppm) (LCL-UCL)	χ^2 (Df)	Slope \pm SE
Essential oil	<i>A. bouvieri</i>	7707.13 ^a (3950.840-135645.468)	24903.72 (8183.569- 3183691.715)	1.48(5)	2.516 \pm 0.808
	<i>T. splendens</i>	4967.50 ^b (3203.262-23203.605)	11633.046 (5555.462-164614.729)	0.40(5)	3.468 \pm 1.004
	<i>G. affinis</i>	7465.93 ^c (3776.745-27751.616)	21659.25 (7120.796-11450761.325)	0.64(5)	2.771 \pm 0.991
	<i>D. indicus</i>	2506.56 ^d (1957.622-5873.229)	4313.126 (2786.461-20242.556)	0.40(5)	5.437 \pm 1.563
β -caryophyllene	<i>A. bouvieri</i>	6335.83 ^a (3830.667-21592.921)	26409.50 (10510.022-260590.887)	2.57(5)	2.067 \pm 0.453
	<i>T. splendens</i>	4418.14 ^b (3097.966-10330.971)	11823.55 (6239.198-56593.581)	2.69(5)	2.998 \pm 0.649
	<i>G. affinis</i>	5424.84 ^c (3313.846-38027.706)	13344.40 (5850.323-368884.286)	3.11(5)	3.278 \pm 1.012
	<i>D. indicus</i>	4524.95 ^d (3144.723-10631.760)	12826.59 (6624.054-62749.488)	3.93(5)	2.832 \pm 0.601
α -Humulene	<i>A. bouvieri</i>	5534.53 ^a (3557.791-14876.619)	22826.33 (9934.008-153027.914)	4.59(5)	2.083 \pm 0.420
	<i>T. splendens</i>	4162.19 ^b (3003.536-8489.781)	11673.61 (6378.430-45232.403)	4.95(5)	2.861 \pm 0.565
	<i>G. affinis</i>	6082.02 ^c (3649.216-27140.183)	19492.24 (7999.169-277482.167)	3.34(5)	2.534 \pm 0.648
	<i>D. indicus</i>	2386.94 ^d (1938.517-3905.869)	4678.51 (3132.171-12403.013)	4.45(5)	4.385 \pm 0.941

Germacrene D	<i>A. bouvieri</i>	5429.94 ^a (3522.213-14125.298)	22253.293 (9828.860-140883.873)	3.76(5)	2.092 ± 0.416
	<i>T. splendens</i>	3926.30 ^b (2541.030-20317.147)	11435.69 (5040.302-292789.666)	8.15(5)	2.760 ± 0.506
	<i>G. affinis</i>	4439.74 ^c (3112.705-10045.497)	12658.45 (6613.556-58371.571)	4.58(5)	2.816 ± 0.586
	<i>D. indicus</i>	2098.80 ^d (1713.703-5700.298)	3255.529 (2240.195-22069.924)	5.83(5)	6.722 ± 1.639
Temephos	<i>A. bouvieri</i>	4.99 ^a (4.534-5.510)	10.93 (9.494-13.085)	0.15(3)	3.767 ± 0.292
	<i>T. splendens</i>	4.85 ^b (4.443-5.312)	9.50 (8.399-11.137)	0.33(3)	4.394 ± 0.352
	<i>G. affinis</i>	5.81 ^c (5.373-6.283)	9.71 (8.744-11.145)	0.07(3)	5.743 ± 0.513
	<i>D. indicus</i>	5.82 ^c (4.869-7.057)	12.35 (9.668-18.528)	3.93(3)	3.925 ± 0.322

LC₅₀ and LC₉₀ - Concentrations needed to kill 50% and 90% of larvae. LCL - Lower confidence limit of 95%. UCL - Upper confidence limit of 95%. χ^2 - Non significant Chi-square ($p < 0.05$). Df - Degree of freedom. Letters in the same column indicate statistical differences (ANOVA and Tukey $p < 0.05$). No mortality was observed in the control.

Table 6. Suitability index / Predator Safety Factor in different predators exposed to the essential oil of *Piper purusanum* and its sesquiterpenes in *Anopheles* larvae

	Predators	<i>An. nuneztovari</i>	<i>An. triannulatus</i>	<i>An. darlingi</i>	<i>An. albitarsis</i>
Essential oil	<i>A. bouvieri</i>	149.36	149.33	154.63	153.98
	<i>T. splendens</i>	96.26	96.25	99.66	99.25
	<i>G. affinis</i>	144.68	144.66	149.79	149.16
	<i>D. indicus</i>	48.57	48.56	50.29	50.08
β -caryophyllene	<i>A. bouvieri</i>	219.53	238.90	252.02	240.35
	<i>T. splendens</i>	153.08	166.59	175.74	167.60
	<i>G. affinis</i>	187.97	204.55	215.78	205.79
	<i>D. indicus</i>	156.78	170.62	179.99	171.65
α -Humulene	<i>A. bouvieri</i>	207.83	167.30	182.29	147.90
	<i>T. splendens</i>	156.29	125.82	137.09	111.22
	<i>G. affinis</i>	228.38	183.85	200.33	162.53
	<i>D. indicus</i>	83.63	72.15	78.62	63.78
Germacrene D	<i>A. bouvieri</i>	167.79	179.14	221.72	173.92
	<i>T. splendens</i>	121.33	129.53	160.32	125.76
	<i>G. affinis</i>	137.19	146.47	181.28	142.20
	<i>D. indicus</i>	64.85	69.24	85.70	67.22
Temephos	<i>A. bouvieri</i>	1.26	1.29	0.95	1.12
	<i>T. splendens</i>	1.23	1.25	0.93	0.88
	<i>G. affinis</i>	1.47	1.50	1.11	1.06
	<i>D. indicus</i>	1.48	1.51	1.11	1.06

Table 7. Suitability Index / Predator Safety Factor in different predators exposed to the essential oil from *Piper purusanum* and its sesquiterpenes in larvae of *Aedes*.

	Predators	<i>Ae. aegypti</i>	<i>Ae. albopictus</i>
Essential oil	<i>A. bouvieri</i>	144.30	180.83
	<i>T. splendens</i>	93.00	116.55
	<i>G. affinis</i>	139.78	175.17
	<i>D. indicus</i>	46.93	58.81
β -caryophyllene	<i>A. bouvieri</i>	211.40	203.78
	<i>T. splendens</i>	147.41	142.10
	<i>G. affinis</i>	180.98	174.46
	<i>D. indicus</i>	150.98	145.54
α -Humulene	<i>A. bouvieri</i>	196.88	191.57
	<i>T. splendens</i>	148.06	144.07
	<i>G. affinis</i>	216.36	210.53
	<i>D. indicus</i>	84.91	82.62
Germacrene D	<i>A. bouvieri</i>	146.91	162.03
	<i>T. splendens</i>	109.18	117.16
	<i>G. affinis</i>	123.46	132.49
	<i>D. indicus</i>	58.36	62.63
Temephos	<i>A. bouvieri</i>	1.55	1.81
	<i>T. splendens</i>	1.51	1.76
	<i>G. affinis</i>	1.80	2.11
	<i>D. indicus</i>	1.81	2.11

Table 8. Acetylcholinesterase activity of the essential oil from *Piper purusanum* and its main sesquiterpenes.

	IC ₅₀ (ppm)	r ²
Essential oil	2.29 ^b (1.562-2.266)*	0.9806
β -caryophyllene	5.37 ^d (3.548-8.728)*	0.9745
α -Humulene	4.36 ^c (2.809-7.172)*	0.9711
Germacrene D	10.91 ^e (7.901-16.040)*	0.9670
Neostigmine	0.77 ^a (0.596-1.022)*	0.9950

*Confidence limit of 95%. Letters in the same column indicate statistical differences (ANOVA and Tukey $p < 0.05$). r² – Linear regression.

Figures

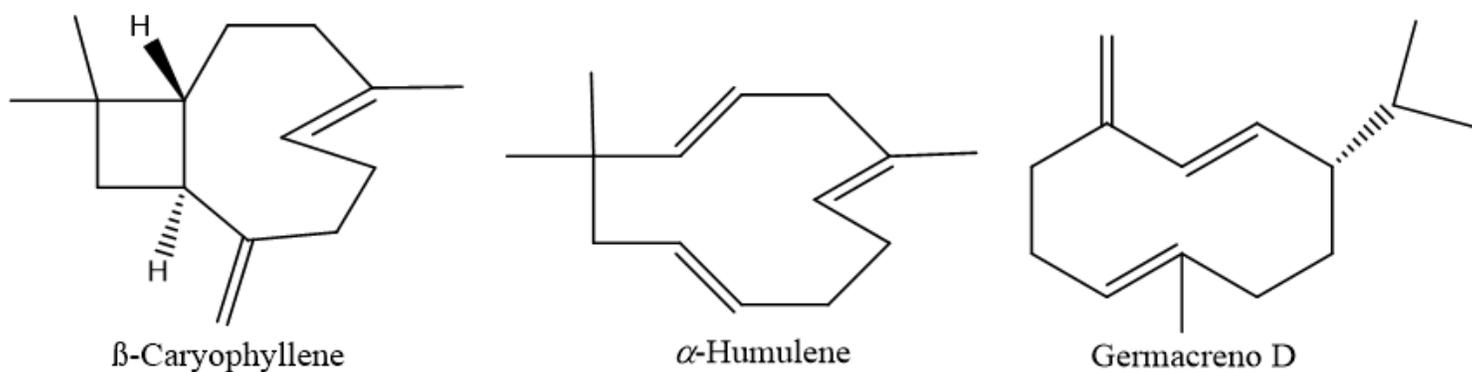


Figure 1

The major sesquiterpenes of essential oil of *Piper purusatum* (ChemBioDraw Ultra).