

Larvicidal activity of hydroalcoholic extracts of *Persea americana* Mill. Seeds against *Aedes aegypti*

Atividade larvica dos extratos hidroalcoólicos das sementes de *Persea americana* Mill. frente

Aedes aegypti

Actividad larvica de extractos hidroalcohólicos de semillas de *Persea americana* Mill. delantero

Aedes aegypti

Received: 07/12/2021 | Reviewed: 07/19/2021 | Accept: 08/01/2021 | Published: 08/06/2021

Ana Paula Muniz Serejo

ORCID: <https://orcid.org/0000-0002-4376-4364>
Universidade Federal do Maranhão, Brazil
E-mail: apsmuniz1@gmail.com

Gustavo Oliveira Everton

ORCID: <https://orcid.org/0000-0002-0457-914X>
Universidade Federal do Maranhão, Brazil
E-mail: gustavooliveiraeverton@gmail.com

Helene do Carmo Castro Lacerda

ORCID: <https://orcid.org/0000-0003-1511-8039>
Universidade Federal do Maranhão, Brazil
E-mail: helene-castro@hotmail.com

Ana Patrícia Matos Pereira

ORCID: <https://orcid.org/0000-0003-4478-4209>
Universidade Federal do Maranhão, Brazil
E-mail: ap.matos11@hotmail.com

João Pedro Mesquita Oliveira

ORCID: <https://orcid.org/0000-0003-1833-9814>
Universidade Federal do Maranhão, Brazil
E-mail: joao-p01@live.com

Thaylanna Pinto de Lima

ORCID: <https://orcid.org/0000-0003-1172-3004>
Universidade Federal do Maranhão, Brazil
E-mail: thaylanna.lima@discente.ufma.br

Maria Giulia Alves Carneiro Felizardo

ORCID: <https://orcid.org/0000-0002-1921-9067>
Universidade Federal do Maranhão, Brazil
E-mail: giullia.73@hotmail.com

Denise Fernandes Coutinho

ORCID: <https://orcid.org/0000-0002-5665-9280>
Universidade Federal do Maranhão, Brazil
E-mail: deniseufma2014@gmail.com

Abstract

This study aimed to evaluate the phytochemical composition of hydroalcoholic extracts from the seeds of *Persea americana* (avocado) and evaluate the larvicidal activity against *Aedes aegypti*. The extracts were obtained by maceration in a 70% PA ethanol extractant solvent in 1:4/1:6/1:8 hydromodules (7 days), with subsequent concentration in a rotaevaporator. The larvicidal activity was performed by the method recommended by the WHO Lethal Concentration 50% against *Aedes aegypti* larvae with statistics by the Probit method. Toxicity to non-target organisms was verified by the *Artemia salina* test. Activity was observed against *Aedes aegypti* larvae with LC₅₀ ranging from 181.72-401.96 mg L⁻¹ with a 95% confidence interval. Finally, it states that the extract of *P. americana* has potential for larvicidal activity and does not present toxicity to target organisms, showing itself to be a sustainable alternative for the control and combat of *Aedes aegypti* larvae.

Keywords: *Persea americana*; Bioativos; Arboviroses; Vetores.

Resumo

Este estudo teve por objetivo avaliar a composição fitoquímica de extratos hidroalcoólicos das sementes de *Persea americana* (avocado) e avaliar a atividade larvica frente *Aedes aegypti*. Os extratos foram obtidos por maceração em solvente extrator etanol 70% P.A. em hidromódulos 1:4/1:6/1:8 (7 dias), com posterior concentração em rotaevaporador. A atividade larvica foi realizada pelo método preconizado pela OMS de Concentração Letal 50%

frente a larvas *Aedes aegypti* com estatística por método Probit. A toxicidade para organismos não-alvos foi verificada pelo teste de *Artemia salina*. Observou-se atividade frente a larvas *Aedes aegypti* com CL_{50} variando de 181,72-401,96 mg L⁻¹ com intervalo de confiança de 95%. Por fim, afirma que o extrato de *P. americana* apresenta potencial de atividade larvicida e não apresenta toxicidade a organismos alvos, mostrando-se uma alternativa sustentável ao controle e combate de larvas *Aedes aegypti*.

Palavras-chave: *Persea americana*; Bioativos; Arboviruses; Vectors.

Resumen

Este estudio tuvo como objetivo evaluar la composición fitoquímica de los extractos hidroalcohólicos de semillas de *Persea americana* (aguacate) y evaluar la actividad larvicida contra *Aedes aegypti*. Los extractos fueron obited por la maceración en etanol del extractor solvente el 70% P.A. en hidromodules 1:4/1:6/1:8 (7 días), con la concentración subsecuente en rotaevaporator. La actividad larvicidal fue realizada por el método del WHO de Concentración Mortal el 50% contra las larvas del *Aedes aegypti* con estadística del Probit. La toxicidad a los organismos de la no-blanco fue verificada por la prueba del *Artemia salina*. La actividad fue observada contra las larvas del *Aedes aegypti* con CL_{50} extendiéndose a partir del 181,72 al 401,96 mg L⁻¹ con un intervalo de confianza del 95%. Por último, se afirma que el extracto de *P. americana* presenta potencial de actividad larvicida y no presenta toxicidad para los organismos diana, demostrando ser una alternativa sostenible al control y combate de las larvas de *Aedes aegypti*.

Palabras clave: *Persea americana*; Bioactivos; Arbovirus; Vectores.

1. Introduction

The *Aedes* mosquito is a vector that transmits arboviruses, has a wide geographic distribution and its expansion is influenced by several factors, including: its behavior, the large size of the urban population, the precariousness of the good proportion of households in relation to the water supply, inadequate disposal of waste, the increase in the number of non-biodegradable containers and inadequate disposal of solid waste. Arboviruses are still considered infections with a great impact on public health, and their prevention and control is one of the main challenges (Moyses&Bonatti, 2021).

The re-emergence of these diseases caused by arboviruses and transmitted by mosquitoes is observed, with emphasis on Dengue, Chikungunya Fever, Yellow Fever and Zika Fever in several countries in the Americas and in other tropical regions. The entry of these viruses in Brazil, a country already endemic for Dengue, represents a major challenge for public health, as they are all susceptible to infections and there are still no specific antivirals, nor vaccines for prevention free from side effects (Viana et al., 2018).

In the absence of dengue drugs and the variable safety and efficacy of the dengue vaccine, Dengvaxia (Thomas&Yoon., 2019), vector control remains the mainstay of Dengue control programs (Chang et al., 2011). Several plants have been used by humanity on all continents to control different diseases and pests, in addition to representing an important source of biologically active natural products, many of which constitute a model for the synthesis of a large number of drugs (Simões et al. 2017).

Therefore, the search for new larvicides from natural resources has always been the criterion for finding alternatives for mosquito control. The advantages of natural insecticides are non-polluting to the environment and are safe for human health. Several natural products have been shown to have mosquito repellent, larvicides, pupicide and ovicidal activities (Shaalán et al., 2005; Nerio et al., 2009; Pavela, 2015; Kishore et al., 2011; Ghosh et al., 2012).

Within this, the search for new pesticides from natural resources has been the criterion for finding alternatives for the control of arbovirus vectors. The advantages of natural biolarvicides include the fact that they are environmentally friendly and safe for human health. Thus, natural products have shown potential in the action of controlling and combating larvicide, pupicide and ovicide (Shaalán et al., 2005; Ghosh et al., 2012; Nerio et al., 2010; Kishore et al., 2011; Pavela, 2015).

Several plants, despite being consumed as functional foods and/or nutraceuticals, are used for bioprospective research, which means the search for chemical products with biological or pharmacological properties that can be used to treat various diseases.

Among these, stands out *Persea americana* Mill. (Lauraceae), being an evergreen tree found and cultivated in the tropical and subtropical region of the world. It's called the avocado fruit and it's edible. In traditional medicine, the plant is reported for a variety of diseases such as heart, skin problems, hypertension, diabetes, bronchitis and diarrhea (Yasir et al., 2010; Dabas et al., 2013). Therefore, considering the importance of finding natural biolarvicides, this study aimed to evaluate the larvicidal activity of hydroalcoholic extracts from *P. americana* seeds against *Aedes aegypti*.

2. Methodology

2.1 Botanical material

Fruits of *Persea americana* were collected from a site located in the Ipem Turu, in the municipality of São Luis, MA in November 2020. These were dried at 40°C in an air-circulation oven and pulverized in a knife mill with moderately coarse powder particle size. . Exsiccates were made with flowering branches and sent to the Ático Seabra Herbarium, at the Center for Biological and Health Sciences at the Federal University of Maranhão, for confirmation of their botanical identification.

2.2 Obtaining hydroalcoholic extracts

To obtain the hydroalcoholic extracts, the maceration technique was used using an ethanol extracting solvent 70% PA in hydromodules of 1:4, 1:6 and 1:8 (m/v), in 70% ethanol. The maceration will be carried out for 10 days, under agitation and filtration (Figure 1).

Figure 1. Filtration processing using the vacuum system to separate the extract solid from the extracting solvent after 10 days of maceration.



Source: Authors (2021).

The resulting extracts, observed in Figure 1, were concentrated in a rotaevaporator (Figure 2) and stored in a refrigerator at 4°C in amber colored flasks (Matos, 2009).

Figure 2. Rotaevaporation of the extracting solvent to concentrate the hydroalcoholic extract.



Source: Authors (2021).

The extracts yields were calculated by gravimetric technique and the physicochemical parameters: density and refractive index were determined according to the techniques recommended by the Brazilian Pharmacopoeia (2019).

2.3 Phytochemical analysis

The extracts were subjected to chemical tests based on the methodology presented by Matos (2009). The tests performed were: Salkowsk test (steroids), Mayer test (alkaloids), flavonoids, glycosides, saponins test, keller kiliani test (cardiac glycosides), ferric chloride test (phenols) and lead acetate test (tannins). And the total phenolic content was quantified through the spectrophotometric method of Folin-Ciocalteu and the total flavonoid through the spectrophotometric method of complexation with aluminum (Lugasi et al., 1998; Oliveira et al., 2009).

2.4 Collection of *Aedes aegypti* eggs

The collection of *Aedes aegypti* eggs was carried out using traps called ovitraps, with the aid of Eucatex palettes. The Eucatex palettes were properly inspected, sanitized and dried before setting up the traps. The traps were installed at various points on the campus of the Universidade Federal do Maranhão, Recanto dos Vinhais (São Luís, MA) and Alemanha (São Luís, MA) under shelter from the sun and rain. The collected eggs were hatched in mineral water and fed until the stage where the experiments were carried out.

2.5 Larvicide activity against *Aedes aegypti*

The tests for larvicidal activity were carried out according to the adapted methodology proposed by Silva (2006). Initially, a 500 mg L⁻¹ stock solution of each of the hydroalcoholic extracts was prepared and diluted in a 2% Tween 80 solution. From this solution, serial dilutions were prepared at concentrations 100-400 mg L⁻¹. At each concentration, 10 larvae were added at the rate of 2 ml per larva.

All tests were performed in triplicate and as negative control a solution made up of 2% Tween 80 was used, and as positive control, a solution of temephos (O,O,O',O'-tetramethyl O,O'-thiodi- p-phenylene bis (phosphorothioate) at 100 ppm, equivalent to the concentration used by the National Health Foundation (FUNASA) for the larvicide control of the vector, in addition to Novaluron (\pm -1-[3-chloro-4-(1-1)- 3-trifluro-2-trifluoromethoxyethoxy) phenyl-3-(2,6-diflurobenzoyl) urea at 0.02 mg L⁻¹, dose adopted by the Ministry of Health, indicated by the WHO in the range of 0.01 to 0.05mg L⁻¹.

After 24 hours the count of live and dead was carried out, and larvae that did not react to touch after 24 hours of the

beginning of the experiment were considered dead. To quantify the efficiency of the extracts, the Probit statistical test (Finney, 1952) was applied.

2.6 Toxicity assessment against *Artemia salina*

This test was performed according to the methodology described by Meyer et al. (1982). To assess the lethality of *Artemia salina* Leach, a stock saline solution of each hydroalcoholic extract was prepared at a concentration of 10,000 mg L⁻¹ and 0.02 mg of Tween 80 (active tension). Aliquots of this were transferred to test tubes and supplemented with saline solution previously prepared up to 5 mL, obtaining in the end concentrations of 1000-10 mg L⁻¹, respectively, where ten larvae in the naupliar stage were transferred to each of the tubes of rehearsal.

For the blank, 5 mL of saline solution was used, for the positive control K₂Cr₂O₇ and for the negative control 5 mL of a 4 mg L⁻¹ solution of Tween 80. After 24 hours of exposure, the count of live larvae was performed, considering dead those that did not move during the observation or with the slight shaking of the flask.

The criterion established by Dolabela (1997) was adopted to classify the toxicity of hydroalcoholic extracts, being considered highly toxic when LC₅₀ ≤ 80 mg L⁻¹, moderately toxic for 80 mg L⁻¹ ≤ LC₅₀ ≤ 250 mg L⁻¹ and slightly toxic or non-toxic when LC₅₀ ≥ 250 mg L⁻¹.

The statistical analysis of the data for the toxicity test was carried out according to the method of Reed&Muench (1938). The intersection point between the curves is the 50% Lethal Concentration (LC₅₀), since at this point the number of surviving animals is equal to the number of dead animals (Colegate; Molyneux, 2007).

3. Results and Discussion

3.1 Phytochemical parameters and phytochemical screening

The physicochemical parameters are important for determining aspects of biological applications and are presented in Table 1.

Table 1. Physicochemical parameters of the hydroalcoholic extract of *Persea Americana*.

Parameter	Density (g mL ⁻¹)	Refractive Index (nD 25°)
EHPA1:8	0.9140	1.345
EHPA1:6	1.0386	1.351
EHPA1:4	0.9440	1.339

Source: Authors (2021).

In a study conducted by Ferrari (2015) evaluating the oil refraction index of *P. americana* seed, it verified a variation of 1.4620 to 1.4625 nD 25°. In another analysis by Jorge (2014) of the pulp oil of this species, 1.4615 nD 25°. Results close to the one found in this search can be found. There is a scarcity of data in the literature regarding the density of extracts from the studied vegetable.

The classes of secondary metabolites identified in the extracts obtained from *Persea americana* are shown in Table 2.

Table 2. Classes of secondary metabolites identified in extracts obtained from the seeds of *Persea americana*.

Hydroalcoholic extracts <i>Persea americana</i>	1	2	3	4	5	6	7	8
EHPA1:8	+	-	+	+	+	+	+	+
EHPA1:6	+	-	+	+	+	+	+	+
EHPA1:4	+	-	+	+	+	+	+	+

Note: 1-Steroids,2-Alkaloids,3-Flavonoids,4-Glycosides, 5-Cardiac Glycosides, 6-Saponins, 7-Phenols,8-Taninos. Source: Authors (2021).

Through the phytochemical study, the following metabolites were identified in the researched extracts: steroids, flavonoids, glycosides, cardiac glycosides, saponins, phenols and tannins, presented in Table 2. The diversity of active compounds present is highlighted, suggesting a high biological potential. Deuschle et al. (2018) evidenced the presence of phenolic compounds and flavonoids in hydroalcoholic extracts of *P. americana* leaves. Athayde et al. (2018) that evaluated the hydroalcoholic extracts of seeds of the studied species registered the presence of phenolic metabolites, tannins and flavonoids.

Table 3 shows the total phenolic content identified in the extracts of *P. americana*.

Table 3. Total phenolic content (mg EAT g⁻¹) quantified for extracts obtained from *Persea americana* seeds.

Extract	Total phenolic content (mg EAT g ⁻¹)	Equation	R ²
EHPA1:8	163.90		
EHPA1:6	126.34	y=0.0586+0.06	0.9998
EHPA1:4	87.07		

Note: EHPA – *Persea americana* hydroalcoholic extract. Source: Authors (2021).

Similar results were described by Rotta et al. (2016) when quantifying the content of phenolics in dehydrated *P. americana* bark and resulted in a quantity of 108.48 ± 1.62 mg GAE g⁻¹). In another study by Athayde et al. (2019) with hydroalcoholic extracts of *P. americana* seeds, the authors verified a total phenolic content quantified in 366.79 ± 5.05 mg EAG g⁻¹.

Table 4 shows the total flavonoid content identified in the extracts of *P. americana*.

Table 4. Total flavonoid content (mg EQ g⁻¹) quantified for extracts obtained from *Persea americana* seeds.

Extract	Total flavonoid content (mg EQ g ⁻¹)	Equation	R ²
EHPA1:8	153.44		
EHPA1:6	120.31	y=0.0033x+0.006	0.9845
EHPA 1:4	90.109		

Source: Authors (2021).

Similar results were also evidenced by Rotta et al. (2016) the flavonoid content of 136.034 ± 1.88 mg EQ g⁻¹ in

dehydrated bark of *P. americana*, there is a result well above that identified in our study. Athayde et al. (2019) when evaluating *P. americana* seed extracts recorded 28.09 ± 0.64 mg EQ g⁻¹.

Gobbo-Neto et al. (2007) claim that there are factors that influence the content of secondary metabolites, among them, one of the most relevant is the period of the year in which the vegetable was collected. Another important factor is the age and development of the plant, as well as the temperature of its habitat, the water supply of the soil in which the plant is grown, exposure to ultraviolet radiation, rainfall and altitude. Therefore, these causes can cause a variation of secondary metabolites in plants.

3.2 Lethality against *Artemia salina* Leach.

Table 5 presents the results regarding the lethality toxicity test against *Artemia salina* Leach.

Table 5. Mortality *Artemia salina* Leach against the action of the hydroalcoholic extract of *Persea americana* in 1:8, 1:6 and 1:4 hydromodule (m/v).

	Accumulated curve intersection log	LC ₅₀ mg L ⁻¹	Classification
EHPA 1:8	2,95	891,30 ^{ab}	Non toxic ^c
EHPA 1:6	2,93	851,10 ^{ab}	Non toxic ^c
EHPA 1:4	2,92	831,80 ^{ab}	Non toxic ^c

Note: ^aLC₅₀ was calculated by Reed&Muench (1938); ^b 95% confidence interval; no dead larvae in the negative control, composed of 4 mg L⁻¹ Tween 80; the positive control, K₂Cr₂O₇, had 100% larval mortality; ^cDolabela (1997). Source: Authors (2021).

The relationship between the degree of toxicity and the mean lethal concentration (LC₅₀) presented for medicinal plant extracts on *Artemia salina* larvae was established by the criteria adopted by Dolabela (1997) for classification of the toxicity of natural products. As observed in Table 5, all extracts obtained presented LC₅₀ above 250 mg L⁻¹, thus being defined as non-toxic, and their application was encouraged.

The nontoxicity profile found in this study is confirmed by Valente (2020) who found a LC₅₀ of 452.57 mg L⁻¹ for the seed extract of *P. americana* against *Artemia salina*. However, toxicity profiles are reported by Amado et al. (2019) when evaluating the toxicity also against *Artemia salina*, but with the ethanolic extract of the bark of the Quintal variety of the *Persea americana* species, where the LC₅₀ found was 204.95 mg L⁻¹. Therefore, it is important to emphasize the use of seed extract in practical applications, aiming at its atoxicity potential, inferring its selectivity to target organisms.

3.3 Lethality against *Aedes aegypti*

Table 6 presents the results regarding mortality *Aedes aegypti* against the action of the hydroalcoholic extract of the seeds of *P. americana*.

Table 6. Mortality *Aedes aegypti* against the action of the hydroalcoholic extract of *Persea americana* in 1:8, 1:6 and 1:4 hydromodule (m/v).

	Log C	%Mortality	LC ₅₀ (mg L ⁻¹) 95% CI	X ²	σ	R ²
EHPA 1:8	2.00	30.0				
	2.30	50.0				
	2.48	70.0	181.76 ^a	0.626	0.465	0.978
	2.60	100.0	(121.95-270.91) ^b			
	2.70	100.0				
	Log C	%Mortality	LC ₅₀ (mg L ⁻¹) 95% CI	X ²	σ	R ²
EHPA 1:6	2.00	10.0				
	2.30	20.0				
	2.48	40.0	401.91 ^a	0.993	0.444	0.978
	2.60	50.0	(273.85-589.85) ^b			
	2.70	60.0				
	Log C	%Mortality	LC ₅₀ (mg L ⁻¹) 95% CI	X ²	σ	R ²
EHPA 1:4	2.00	40.0				
	2.30	50.0				
	2.48	60.0	181.72 ^a	0.265	0.963	0.978
	2.60	100.0	(83.02-397.76) ^b			
	2.70	100.0				

Note: ^aLC₅₀ was calculated by Probit analysis (Finney, 1952); ^b 95% confidence interval; no dead larvae in the negative control, composed of 2% DMSO solution; the positive control, 1 mg L⁻¹ temephos, had 100% larval mortality. Source: Authors (2021).

As shown in Table 6, it is observed that the lowest LC₅₀ was observed for EHPA1:4, quantified at 181.72 mg L⁻¹. However, an action similar to EHPA1:8 is observed with a statistically similar LC₅₀ of 181.76 mg L⁻¹.

To analyze the efficiency of larvicidal activity of natural products, some studies suggest some criteria, as there is still no standard concentration range for determining the efficiencies of natural products. It should be considered that these criteria are related to the time of exposure to the bioproducts and the origin of the larvae, which can change the LC₅₀ values. According to Komalamisra et al. (2005), LC₅₀ between 100 and 750 mg L⁻¹ are effective, up to 48 hours of exposure. Thus, the LC₅₀ obtained for *P. americana* seed extracts obtained in this study can be correlated to effective alternatives in the control of *Aedes aegypti*.

The larvicidal action observed was also described in the study by Torres et al. (2014) in locations in the Philippines, in which the authors used two extracting solvents (ethanol and hexane), and LC₅₀ was observed in *P. americana* seed extracts, respectively, at intervals from 16.48 mg L⁻¹ to 20.61 mg L⁻¹ and 9.82 mg L⁻¹ to 13.39 mg L⁻¹. Already in larvicidal trials performed by Agrela et al. (2014), in which methanol was adopted as solvent in *P. americana* seed extracts, the LC₅₀ were recorded for Ruckerfeller and Mario Briceno Iragorry strains, respectively, 5.7 mg L⁻¹ and 9.9 mg L⁻¹. In a research carried out by Louis et al. (2020), in which the rind of the fruit of the same plant species was subjected to methanol extract, the LC₅₀ detected for 3rd stage larvae was 7.12 mg L⁻¹.

Comparing with the LC₅₀ results identified in the present study, it is noted that larvicidal activity was evidenced, however with a lower lethality potential, requiring a higher extract concentration to obtain the death of 50% of the larvae.

It is noteworthy that the results obtained with plant extracts may vary according to the period of collection, temperature, type of soil cultivated, plant species and others. For this reason, the isolation of the active compound from extracts is essential for standardization as a method of larval control (Gupta et al., 2011).

The solvent used can directly contribute to the variation of larvicidal activity, since it has been shown that the extraction of active biochemicals from plants requires the polarity of the solvents used (Ghost et al., 2012). The favorable results obtained encourage the continuation of the study, aiming at the isolation of the active principle and, mainly, ways that enable its practical use for the control of *Aedes aegypti*.

It was not yet possible through the study to specify which component or which components are responsible for larvicidal activity, because the isolation of such has not yet been verified. It is also necessary to perform the chemical characterization by chromatographic techniques of the extracts studied. Thus, it will be feasible to identify the metabolites to be attributed such larvicide potentials, being possible to generate bioproducts with bioprospecting activity for development of larvicide agents of plant origin.

4. Conclusion

The seeds of the species *Persea americana* presented a heterogeneity of secondary metabolites, encouraging future studies with other biological potentials. The tested extracts show activity against *Aedes aegypti* larvae, still revealing an atoxicity in the bioassay with *Artemia salina* against the non-target organism, showing selectivity against target organisms. Finally, the potential for using this product to control the vector responsible for the propagation of important arboviruses with high morbidity and mortality is confirmed and efficient, and should be explored in its control and combat.

References

- Amado, D. A. V., Helmann, G. A. B., Detoni, A. M., Carvalho, S. L. C. D., Aguiar, C. M. D., Martin, C. A., & Cottica, S. M. (2019). Antioxidant and antibacterial activity and preliminary toxicity analysis of four varieties of avocado (*Persea americana* Mill.). *Brazilian Journal of Food Technology*, 22.
- Agrela, I. F., Hidalgo, Y., & Herrera, F. (2014). Efecto larvicida de extractos metanólicos obtenidos de semillas y hojas de *Persea americana* (Laurales: Lauraceae)(aguacate) sobre *Aedes aegypti* (Diptera: Culicidae). *Boletín de Malariología y Salud Ambiental*, 54(2), 199-207.
- Chang, M. S., Christophel, E. M., Gopinath, D., & Abdur, R. M. (2011). Challenges and future perspective for dengue vector control in the Western Pacific Region. *Western Pacific surveillance and response journal: WPSAR*, 2(2), 9.
- Colegate, S. M., & Molyneux, R. J. (Eds.). (2007). *Bioactive natural products: detection, isolation, and structural determination*. CRC press.
- Deuschle, V. C., Brusco, I., Piana, M., Faccin, H., de Carvalho, L. M., Oliveira, S. M., & Viana, C. (2019). *Persea americana* Mill. crude extract exhibits antinociceptive effect on UVB radiation-induced skin injury in mice. *Inflammopharmacology*, 27(2), 323-338.
- Dolabela, M.F. (1997) Triagem in vitro para atividade antitumoral e anti Trypanossoma cruzi de extratos vegetais, produtos naturais e substâncias sintéticas. Dissertação (Mestrado), Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais-UFMG, Belo Horizonte, 128 p.
- Dabas, D., M Shegog, R., R Ziegler, G., & D Lambert, J. (2013). Avocado (*Persea americana*) seed as a source of bioactive phytochemicals. *Current pharmaceutical design*, 19(34), 6133-6140.
- Farmacopeia Brasileira. (5a ed.), ANVISA, 2010.
- Ferrari, R. A. (2015). Nota Científica: Caracterização físico-química do óleo de abacate extraído por centrifugação e dos subprodutos do processamento. *Braz. J. Food Technol.*, 18, 79-84.
- Finney, D. J. (1952). *Probit analysis: a statistical treatment of the sigmoid response curve*. Cambridge university press, Cambridge.
- Gobbo-Neto, L., & Lopes, N. P. (2007). Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. *Química nova*, 30(2), 374-381.
- Ghosh, A., Chowdhury, N., & Chandra, G. (2012). Plant extracts as potential mosquito larvicides. *The Indian journal of medical research*, 135(5), 581.

- Gupta, L., Deshpande, S., Tare, V., & Sabharwal, S. (2011). Larvicidal activity of the α -amylase inhibitor from the seeds of *Macrotyloma uniflorum* (Leguminosae) against *Aedes aegypti* (Diptera: Culicidae). *International Journal of Tropical Insect Science*, 31(1-2), 69-74.
- Jorge, T. D. S. (2014). Avaliação reológica do óleo de abacate (*Persea americana* mill) e estudo da estabilidade sob condições de aquecimento e armazenamento à temperatura ambiente.
- Kishore, R. K., Halim, A. S., Syazana, M. N., & Sirajudeen, K. N. S. (2011). Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. *Nutrition research*, 31(4), 322-325.
- Komalamisra, N., Trongtokit, Y., Rongsriyam, Y., & Apiwathnasorn, C. (2005). Screening for larvicidal activity in some Thai plants against four mosquito vector species. *Southeast Asian Journal of Tropical Medicine and Public Health*, 36(6), 1412.
- Louis, M. L. M., Pushpa, V., Balakrishna, K., & Ganesan, P. (2020). Mosquito larvicidal activity of Avocado (*Persea americana* Mill.) unripe fruit peel methanolic extract against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi*. *South African Journal of Botany*, 133, 1-4.
- Lugasi, A., Dworschák, E., Blazovics, A., & Kery, A. (1998). Antioxidant and free radical scavenging properties of squeezed juice from black radish (*Raphanus sativus* L. var *niger*) root. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 12(7), 502-506.
- Matos, F. D. A. (2005). *Introdução à fitoquímica experimental*. edições UFC.
- McLaughlin, J. L. (1982). Brine shrimp: a convenient general bioassay for active constituents. *Planta Med*, 45, 31-32.
- Moyses, V., & Bonatti, A. F. (2021). A educação em saúde e a intersectorialidade como estratégia de prevenção e controle das arboviroses. *The Brazilian Journal of Infectious Diseases*, 25, 101175.
- Nerio, L. S., Olivero-Verbel, J., & Stashenko, E. (2010). Repellent activity of essential oils: a review. *Bioresource technology*, 101(1), 372-378.
- Oliveira, A. C. D., Valentim, I. B., Goulart, M. O. F., Silva, C. A., Bechara, E. J. H., & Trevisan, M. T. S. (2009). Fontes vegetais naturais de antioxidantes. *Química Nova*, 32, 689-702.
- Pavela, R. (2015). Essential oils for the development of eco-friendly mosquito larvicides: A review. *Ind Crops and Prod*, 76, 174-187.
- Rotta, E. M., de Moraes, D. R., Biondo, P. B. F., dos Santos, V. J., Matsushita, M., & Visentainer, J. V. (2016). Use of avocado peel (*Persea americana*) in tea formulation: a functional product containing phenolic compounds with antioxidant activity. *Acta Scientiarum. Technology*, 38(1), 23-29.
- Reed, L.J. e Muench, H. (1938) Um método simples de estimar 5% de endpoints. *American Journal of Hygiene*, 27, 493-497.
- Shaaan, E. A. S., Canyon, D. V., Bowden, B., Younes, M. W. F., Abdel-Wahab, H., & Mansour, A. H. (2006). Efficacy of botanical extracts from *Callitris glaucophylla* against *Aedes aegypti* and *Culex annulirostris* mosquitoes. *Tropical Biomedicine*, 23, 180-185.
- Simões, C. M. O., Schenkel, E. P., de Mello, J. C. P., Mentz, L. A., & Petrovick, P. R. (2017). *Farmacognosia: do produto natural ao medicamento*. Artmed Editora.
- Silva, H. H. G., Silva, I. G. D., Elias, C. N., Lemos, S. P. S., & Rocha, A. P. (1995). Idade fisiológica dos ovos de *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) (Diptera, Culicidae). *Revista Pat. Trop.* 24(2), 269-273.
- Thomas, S. J., & Yoon, I. K. (2019). A review of Dengvaxia®: Development to deployment. *Human vaccines & immunotherapeutics*, 15(10), 2295-2314.
- Torres, R. C., Garbo, A. G., & Walde, R. Z. M. L. (2014). Larvicidal activity of *Persea americana* Mill. against *Aedes aegypti*. *Asian Pacific journal of tropical medicine*, 7, S167-S170.
- Valente, G. C. (2020). Caracterização e análise comparativa dos óleos extraídos da semente e da polpa do abacate (*Persea americana* Mill.) utilizando diferentes métodos de extração.
- Viana, L. R. D. C., Pimenta, C. J. L., Araújo, E. M. N. F. D., Teófilo, T. J. S., Costa, T. F. D., & Costa, K. N. D. F. M. (2018). Reemerging arboviruses: clinical-epidemiological profile of hospitalized elderly patients. *Revista da Escola de Enfermagem da USP*, 52.
- Yasir, M., Das, S., & Kharya, M. D. (2010). The phytochemical and pharmacological profile of *Persea americana* Mill. *Pharmacognosy reviews*, 4(7), 77.