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作品名稱 **EVALUATION OF THE SURFACE TENSIO, LARVICIDAL AND ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS FROM THE LEAF OF THE ARAÇÁ (Psidium guineense Sw.) TO COMBAT THE PROLIFERATION OF THE Aedes aegypti MOSQUITO IN STILL WATER CONTAINERS**

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ABSTRACT

The *Aedes aegypti mosquito* is one of the main transmitters of viral diseases in countries close to the equator. This vector promotes a series of generalized endemics that are difficult to control and prevent in these regions. Furthermore, the presence of bacteria in the environment favors the proliferation of mosquito larvae, which increases the probability of *Aedes aegypti* reproductive success. The Araçzeiro (*Psidium guineense* Sw.) is a plant present throughout the Brazilian Atlantic Forest and has in its composition, especially in the leaves, several substances that can be used to solve problems. Thus, we sought to verify the activity of flavonoids and polyphenols in terms of their antibacterial potential and the performance of saponins in their larvicidal potential, as well as surfactant, in order to prevent the accommodation of the mosquito in the water at the time of egg deposition and larvae respiration. The saponins were extracted from the araçazeiro leaf using a hydroalcoholic solvent and the flavonoids/polyphenols using methanol, the latter being subsequently rotaevaporated to maintain the non-toxic nature of the extract. Through the aqueous extracts, the content of total saponins by UV-VIS spectrophotometry, surfactant activity, larvicidal activity and toxicity were determined. In relation to the ethanolic extracts, the content of polyphenols and total flavonoids by UV-VIS spectrophotometry and high performance liquid chromatography (HPLC), antibacterial activity and toxicity were determined. The results showed that the aqueous extract has a satisfactory amount of saponins, as well as a surfactant potential due to the formation of foam and larvicidal activity in the two highest concentrations of the extracts. Ethanol extracts showed phenolic acids, especially gallic and ellagic acid, and flavonoids, especially catechin and quercetin, and antibacterial activity in most of the worked concentrations. Both extracts (aqueous and ethanolic) showed a dominant non-toxic character, which favors their use without risk to the environment, having an alternative and sustainable potential for controlling the proliferation of the *Aedes aegypti mosquito.*

1. INTRODUCTION

The *Aedes aegypti mosquito* is an insect characteristic of tropical regions and is predominantly found in urban areas, especially in places that have water deposits and temporary collections. (NELSON, 1986). Diseases from the *Aedes aegypti mosquito* have been reported in Brazil since 1846. However, only a century later, the mosquito gained notoriety for causing frequent epidemics in the country's largest cities. From the 2000s, the mosquito gained worldwide prominence with the various seasonal outbreaks of diseases in various parts of the world, especially in the American continent. (BRAGA et al., 2007).

The female mosquito has a hematophagous style of nutrition, which is necessary for the reproduction process. Females are agile and can move quickly in search of new hosts in the environment. After each oviposition on the surface of the liquid or on the walls of standing water containers, females seek hosts as quickly as possible and this behavior is what promotes epidemiological emergencies (NATAL, 2002). Generally, epidemics caused by the mosquito are the following diseases: zika virus, dengue and chikungunya, which pose serious risks to society. (TAUIL, 2006). Another important factor is related to the development of *Aedes aegypti larvae,* which rely on the surface tension of the water to obtain oxygen, through a structure called a siphon.

Regarding dengue, for example, according to the Ministry of Health, it is estimated that there are more than 50 million infections in the world, and dengue is responsible for an average of approximately one thousand deaths in Brazil. This rate varies from year to year, however, epidemiological surveillance bulletins indicate that dengue is in a growing phase, despite the underreporting of cases in recent years, reviving a latent problem in Brazilian society. In addition, the epidemiological bulletin, issued by the health surveillance secretariat, in February 2022, pointed out that several states, such as Rondônia, Tocantins, Bahia, Maranhão, Alagoas, Rio Grande do Sul, Goiás and the Federal District presented variations of more than than 200% of cases related to the Zika virus.

Bacteria are extremely important for *Aedes aegypti* from the larval stage to the adult stage. These microorganisms act in the metabolic processes of the

mosquito itself, especially in obtaining biochemical resources, through a complex network that forms the microbiota of the mosquito. (OSEI-POKU et al., 2012). This harmonic relationship can be demonstrated through the action of bacteria in obtaining essential heme prosthetic groups for the mosquito, such as iron. The microbiota of these insects is constantly changing, mainly from the aquatic to the terrestrial stage. However, they are essential for the development of the mosquito in its life cycle. (JUPATANAKUL et al., 2014). In addition, the bacteria in the environment not only favor the proliferation of the mosquito, but also pose a risk of infection for the population.

The Araçazeiro (*Psidium guineense* Sw.) is a bushy tree that reaches an average of 4.5 meters in height and is present in a large part of the Brazilian territory, especially in the Northeast and Southeast, with other outbreaks in the American continent. Its branches are tortuous, smooth stem, simple leaves with salient innervations, slightly wavy margins and are plants adapted to environmental stress (pollution, parasites, etc.), which favors the large production of secondary metabolites. Secondary metabolites can be used for adverse purposes, as they have applicable bioactive activity. Therefore, this species of araçazeiro has always been greatly exploited for its properties, such as the use of its roots and leaves in folk medicine and its wood for the manufacture of resistant cork. Its fruits have a large amount of vitamin C, mineral salts and fiber. (BEZERRA et al., 2016). Among the various secondary metabolites, saponin, flavonoids and phenolic acids stand out in their pharmacological activity.

Saponins or saponosides are a group of diverse glycosides that have an amphiphilic structure and, therefore, are powerful surfactants, detergents and surfactants. Its molecular properties also allow the cell membranes of simple organisms to be disrupted, having great larvicidal and antimicrobial potential (FERREIRA, 2018). In addition, saponins are used by natural medicine, as this compound has anti-inflammatory, analgesic, expectorant, antioxidant and cholesterol-lowering activities (SPARG et al., 2004). Saponins, because they have detergent activity, are capable of producing persistent foam after agitation, which validates several qualitative observation methods related to surfactant activity. (CARDOZO et al., 2014).

Flavonoids are one of the largest groups of substances derived from plant secondary metabolism. These phytochemicals have a low molecular weight and usually have aromatic rings in their molecular composition (PRADO et.al., 2018). In contrast, phenolic acids represent a smaller group, which are divided into hydroxybenzoic acids and hydroxycinnamic acids. (MANACH et al., 2004; D'ARCHIVI et al., 2007). The antibacterial activity of flavonoids and polyphenols comes from the hydroxyl groups, which have very high activity with protein groups, as well as inhibiting specific enzymes and disrupting their metabolic pathways. (ALCARÁZ et al., 2000; ÀVILA et al., 2008).

Therefore, it appears that the surface tension and the bacterial microorganisms present in the medium are determining factors for the proliferation of *Aedes aegypti* in containers containing still water. In addition, the presence of saponins, polyphenols and flavonoids in the leaves of the Araçazeiro tree, from secondary metabolism, have the potential to mitigate the impacts caused by the mosquito.

1. 1 Objectives

1.1.1 General

To evaluate the surface-active, larvicidal and antibacterial potential of secondary metabolites present in plant extracts from the leaf of the Araçazeiro (*Psidium guineense* Sw) to minimize the proliferation of the *Aedes aegypti mosquito* in standing water containers.

1.1.2 Specifics

- Determine the content of saponin, flavonoids and polyphenols in the extracts, using spectrophotometry;
- Characterize and quantify phenolic acids through analytical methods such as High Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD).
- Carry out chemometric analyzes with the data obtained;
- Evaluate the surfactant potential of aqueous extracts;
- Verify the antibacterial activity of ethanolic extracts;
- Investigate the toxicity of the obtained extracts.

2 . METHODOLOGY

2.1 Plant materials

The leaf samples of the araçazeiro (*Psidium guineense* Sw.) were collected in September 2021, in the morning period, in 5 different neighborhoods of the city of Salvador, Bahia, Brazil, which were cataloged according to table 1. leaves were submitted to an experimental research.

Table 1. Identification of sampling regions.

PLACES	ACRONYMS
sprouts	BRT
kabula	CAB
cajazeiras	CAJ
São Cristóvão Park	PSC
New Horizon	NH

Source: the Author (2021).

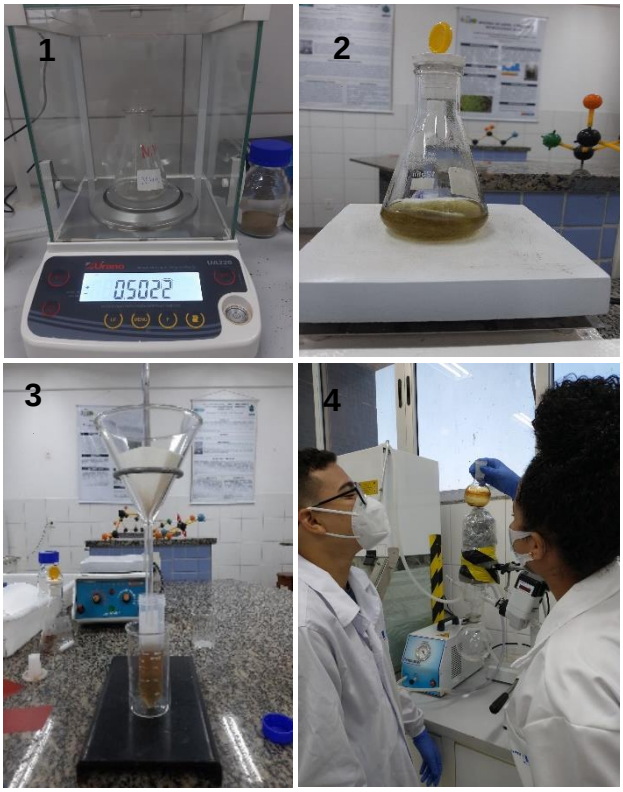
The leaves were cleaned with distilled water and dried in an oven for approximately 6 hours at a temperature of 50°C. After that, he crushed them in a knife mill and packed them in moisture-free and airtight containers (SAUTHIER et.al., 2019). After collection, some samples from the 5 locations were separated for the preparation of specimens of leaves, flowers and fruits. The specimens were sent to the Catholic University of Salvador, Salvador -BA, for taxonomic determination of the species.

2.2 Preparation of extracts

2.2.1 Aqueous extracts (saponins)

For the saponin extraction process, the methodology by DOS SANTOS, et al. (2011) with adaptations. The extraction was performed with 0.5000 g of the sample added to 30 mL of 95% ethanolic solvent. Then, the extraction took place under constant agitation at 100 rpm and 25 °C for 18 min for each location, using a stirrer plate with a magnetic bar (Logen). The extract was filtered and concentrated in a rotary evaporator at 80 °C. After this process, the extracts were placed in 1.5 mL Eppendorf-type microtubes at low temperature and without exposure to light. The simplified steps were shown in figure 1.

Figure 1. General steps for obtaining extracts



Caption: 1: weighing; 2: extraction; 3: filtration; 4: rotary evaporation.

Source: the Author, 2021.

2.2.2 Ethanol extracts (phenolic acids and flavonoids)

For the extraction process of phenolic acids and flavonoids, the methodology of SAUTHIER et al. (2019) with adaptations. The extraction took place with 0.5000 g of the sample added to 30 mL of methanol acidified with 10 µL of concentrated HCl. Then the extraction took place under constant agitation at 100 rpm and 25 °C for 30 min for each location. The extract was filtered and concentrated in a rotary evaporator at 60°C and 15 rpm, and the resulting material was resuspended with 1.5 mL of ethanol. After this process, the extracts were placed in 1.5 mL Eppendorf-type microtubes at low temperature and without exposure to light. The general steps were demonstrated in figure 8.

2.3 Biochemical determinations

2.3.1 Total saponin content (TSC)

The total saponin content (TSC) was determined by the methodology adapted from cobalt chloride in triplicate for each locality (VIGO et al., 2003). 1 mL of aqueous plant extract previously diluted in 100 mL of water, 1 mL of cobalt

chloride at 2.0% (w/v) in distilled water and 1 mL of concentrated sulfuric acid were used. After 20 min, readings were taken at 284 nm, using a UV-Vis spectrophotometer (600 plus - Femto). The calibration curve was generated using Sigma saponin and the coefficient of determination (R^2) was equal to 0.9974. Results were expressed as saponin equivalents per 100 g dry weight (SP 100 g^{-1} DW).

2.3.2 Total flavonoid content (TFC)

The total flavonoids (TFC) content was determined by the methodology adapted from aluminum chloride in triplicate (DOS SANTOS et al., 2017). 20 µL of ethanolic plant extract, 3.0 mL of 2.0% (w/v) hydrated aluminum chloride solution in ethanol were used and measured with ethanol up to 5.0 mL. After 30 min, readings were performed at 415 nm, using a UV-Vis spectrophotometer (SP-22 Biospectro, Brazil) . The calibration curve was generated using Merck quercetin and the coefficient of determination (R^2) was equal to 0.9920. Results were expressed as quercetin equivalents per 100 g of dry mass (QE 100g^{-1} DW).

2.3.3 Total polyphenol content (TPC)

The total polyphenols (TPC) content was determined by the methodology adapted from the Folin-Ciocalteu chromogenic reagent in triplicate (DOS SANTOS et al., 2017). 500µL of Folin-Ciocalteu reagent was added to 20µL of ethanolic extract, 400µL of sodium carbonate 7.5% (w/v), and calibrated with distilled water to 10 mL. After 30 minutes, the reading was performed in a UV-VIS spectrophotometer (SP-220 Biospectro, Brazil) at a wavelength of 760 nm. The results were calculated using a gallic acid calibration curve and the coefficient of determination (R^2) was equal to 0.9970. Results were expressed as gallic acid equivalents per 100 g dry mass (GAE100 g^{-1} DW).

2.3.4 High Performance Liquid Chromatography (HPLC – UV)

The biochemical profile was determined by high performance liquid chromatography (HPLC), following the methodology adapted from DOS SANTOS et al., 2017. A liquid chromatography system (Shimadzu Scientific Instruments, Japan) from the State University of Bahia (UNEB) was used.), which consists of

a high-pressure quaternary pump (LC-20AD, Shimadzu), degassing aspirator, manual high-pressure injector valve (20 µL injection loop), and a photodiode array detection system (PDA) (SPD-20A, Shimadzu). The chromatograph was equipped with a “Licrhorspher” column RP 18 (Agilent), 5 µm, 4.6 × 250 mm, controlled by the LC-System program.

The determination and qualitative analyzes were performed with standard spectra for each retention time. Ethanolic extracts were tested at different wavelengths (nm): 260 for protocatechuic acid, ellagion acid; 272 for gallic acid, syringic acid and chrysin; 280 for catechin, vanillin, transcinnamic acid and naringenin; 310 for p-coumaric acid; 330 for chlorogenic acid, caffeic acid, ferulic acid and sinapic acid; 360 for rutin, quercetin and kaempferol.

The separation column was configured to operate at 42 °C with analytical solvents constituting a binary grid for the elution mixture: methanol and 1% acetic acid (v/v). The run was performed in 43 min at a continuous flow rate of 0.8 mL/min. The program's gradient adjustment followed: 0–10 min, 100% A; 10–20 min, 70% A; 20–30 min, 10% A; 30–37 min, 70%A and 37–43 min, 100%.

2.3.5 Data processing

From the data generated, the information related to the spectrophotometric and chromatographic analyzes was used to organize the chemometric analyses. Therefore, using the Statistica software, version 7.0, Principal Components Analysis (PCA) and Hierarchical Clustering Analysis (HCA) were performed.

2.4 Efficiency tests

2.4.1 Evaluation of surfactant activity

The potential of the extract for a surface-active agent (detergent) was evaluated by the methodology adapted from the height of the foam column (SOUZA et al., 2004). 1 mL of concentrated aqueous extract was diluted in 100 mL of distilled water. Then , 10 mL of this mixture was placed in test tubes and stirred constantly and vigorously for 5 seconds. After 15 minutes, persistent foam was measured in millimeters (mm) using a caliper. This test was performed in triplicate for each location and the results expressed as the average of the values.

2.4.2 Evaluation of larvicidal activity

The larvae used in the bioassays were supplied by endemic agents of the institution: Centro de Controle de Zoonoses (CCZ) of the city of Salvador in hermetic tubes. The larval stages used were 1 and 2, defined through morphological identification (larvae size and pecten structure). The evaluation of the larvicidal activity was obtained by a relation between the concentration of the extract and mortality (%) of the larvae of the *Aedes aegypti mosquito* after 24 hours of the application of the extract. Four concentrations (332 Kg/m ^{3} ; 249 Kg/m ^{3} ; 166 Kg/m ^{3} ; 83 Kg/m ^{3}) were used in the tests. 500 uL of applied extract was added to 5 mL of water in airtight tubes. 2 larvae were used for each test. The experiment took place in an air-conditioned room, with a temperature of 27°C. (CUNHA, 2014).

2.4.3 Evaluation of antibacterial activity

The methodology adopted was the adapted Kirby-Bauer method, known as the disc diffusion method (NOVAIS et al., 2003). The experiments were carried out with ethanolic plant extracts (phenolic acids and flavonoids) from the five locations. The sterilized Muller-Hinton Agar medium was added to the Petri dishes, then a bacterial suspension was inoculated with the aid of a Drigalski loop, containing the *Escherichia coli strain* , obtained from the Catholic University of Salvador (figure 2). Dry and sterile diffusion discs (5 mm) were placed in Petri dishes and subsequently impregnated with 13 µL of the extract to be tested. The procedure was performed with four concentrations (332 Kg/m ^{3} ; 249 Kg/m ^{3} ; 166 Kg/m ^{3} ; 83 Kg/m ^{3}) for each extract from different locations, as shown in Table 2. All plates were placed in the bacteriological oven for 24 hours at a temperature close to 37º C. After this process, the size of the inhibition halos in millimeters (mm) was measured. The control was carried out with the antibiotic gentamicin 10 µg/disc.

Figure 2. Inoculation of petri dishes and growth in a bacteriological oven.

Source: the Author, 2021.

Table 2. Identification and concentration of impregnated extracts

Petri dish	Identification of plate extracts	Extract concentration (Kg/m ^{3})
Control	---	---
1	BRT/CAB	332
two	PSC/CAJ	332
3	NH	332
4	BRT/CAB	249
5	PSC/CAJ	249
6	NH	249
7	BRT/CAB	166
8	PSC/CAJ	166
9	NH	166
10	BRT/CAB	83
11	PSC/CAJ	83
12	NH	83

BRT: Brotas; CAB: Kabula; CAJ: Cajazeiras; NH: New Horizon; PSC: São Cristóvão Park.

Source: the Author, 2021.

2.4.4 Evaluation of extract toxicity

The toxicity of the extracts was evaluated through the mean mortality of *Artemia salina* . The crustacean was cultivated using the ecosystem simulation methodology (BUENO & PIOVEZAN, 2015; DUAVY et al., 2012). Thus, a saline solution was prepared with a concentration of 30 kg/m ^{3} with mineral water. The pH of the medium was adjusted to a value between 8-9 with a 0.1 mol/L sodium hydroxide (NaOH) solution. The medium temperature was kept constant at 25º C and the *A. salina eggs were placed* in the hatching container. In addition, it was

necessary to keep the environment under constant white light. The nauplii were fed with spirulina solution (phytoplankton).

After hatching the eggs, the test was conducted with the four different concentrations as shown in Table 3, in order to define the average rate of dead *brine shrimp* after the 24-hour period. 500 uL of the extracts were used as a fixed volume in a test tube containing 5 mL of saline solution with the microcrustaceans. Aqueous extracts (saponin) and ethanol extracts (phenolic acids and flavonoids) were evaluated. Toxicity was defined by the percentage (%) of mortality (non-toxic < 50% > toxic).

Table 3. Evaluated extract concentrations and volume of saline solution

Samples	Extract concentration (Kg/m ^{3})	Volume of saline solution (mL)
Control	---	5
1	332	5
two	249	5
3	166	5
4	83	5

Source: the Author, 2021.

3. RESULTS AND DISCUSSION

The samples were morphologically determined, demonstrating that they belonged to the species *Psidium guineense* Swartz. The results below were discussed comparing the analyzes of the specific analytes together in relation to the 5 collection locations.

3 .1 Biochemical determinations

3.1.1 Total saponin content (TSC)

Through spectrophotometric analyses, it was possible to determine the concentration in mg/100g for each extract, as well as the standard deviation in triplicate, as shown in table 4.

Table 4. Results of total saponin content by location in mg/100g

Samples	Average (mg/100g) ± DPR
PSC	28.72 ± 3.03
NH	28.48 ± 1.16
BRT	15.88 ± 4.94
CAB	25.70 ± 2.88
CAJ	16.43 ± 2.31

BRT: Brotas; CAB: Kabula; CAJ: Cajazeiras; NH: New Horizon; PSC: São Cristóvão Park.

Source: the Author, 2021.

The highest concentrations were obtained in the PSC and NH extracts, according to table 4. The difference in concentration found in the samples may be related to the characteristics of the collection medium, since the concentration of secondary metabolites has a direct response to environmental stresses, such as such as the incidence of light, temperature, humidity, water, pollutants, among others (BORGES et al., 2017). Therefore, the concentration of saponin in the PSC and NH extracts of the samples collected by the roadside, in a location with a high concentration of pollutants and high temperature, was already expected. On the other hand, the extracts from the BRT and CAJ sample had lower saponin contents. These results corroborate the fact that the different stresses, which the plants are submitted, can significantly influence the content of secondary metabolites, because although the BRT sample was collected in a region with less influence of pollutants and the CAJ sample in a region with opposite characteristics, the results were similar. It can be inferred, then, that biotic factors, such as the actions of fungi or bacteria, or abiotic ones, such as soil and temperature, directly influence the synthesis of secondary metabolites. The presence of saponins may favor its use in combating *Aedes aegypti* , as this group of substances has detergent (CASTEJON, 2011) and larvicidal (ANDRADE, et.al., 2021) potential. This approach will be discussed in topic: 4.2.1; 4.2.2, respectively.

3.1.2 Total flavonoid content (TFC)

The results showed that the total complexed flavonoids present in the ethanolic extracts were determined. Therefore, it was possible to determine the

mean concentration in mg/100g for each extract, as well as the standard deviation of the triplicate, as shown in Table 5.

Table 5. Results of flavonoid content by location in mg/100g

Samples	Average (mg/ 100g) ± DPR
PSC	737.39 ± 0.99
NH	724.79 ± 0.03
BRT	410.08 ± 0.02
CAB	333.72 ± 0.01
CAJ	727.75 ± 0.49

BRT: Brotas; CAB: Kabula; CAJ: Cajazeiras; NH: New Horizon; PSC: São Cristóvão Park.

Source: the Author, 2021.

The results shown in Table 5 indicated that the extracts from the PSC, NH and CAJ locations had the highest concentrations. This concentration difference showed better antibacterial activity results, as shown in Table 11. The concentrations (mg/100g) demonstrate that the locational environmental stress that the plants suffered may have influenced the greater production of secondary metabolites, according to BORGES et al. ., (2017). The PSC, NH and CAJ samples were collected at the side of the road, therefore, it can be inferred that this factor could have contributed directly or indirectly to the higher amount of flavonoids per sample. Therefore, it can be stated that the optimization of obtaining compounds from the secondary metabolism of this species of araçazeiro is linked to biological, climatic and locational patterns. According to AQUINO et.al., (2016), flavonoids have antibacterial activity, therefore, it can be inferred that the presence of these metabolites has the potential to act as a bactericidal agent. This approach will be better discussed in topic: 4.2.1.

3.1.3 Total polyphenol content (TPC)

It was possible to determine the concentration in mg/100g for each extract, as well as the standard deviation in triplicate, as shown in Table 6. The concentration of total polyphenols in these samples corroborated the antibacterial profile of the extract, as shown in Table 11.

Table 6. Results of phenolic acid content by location in mg/100g

Samples	Average (mg/ 100g) ± DPR
PSC	71.97 ± 0.88
NH	76.00 ± 2.21
BRT	59.54 ± 1.06
CAB	60.64 ± 4.55
CAJ	64.29 ± 4.51

BRT: Brotas; CAB: Kabula; CAJ: Cajazeiras; NH: New Horizon; PSC: São Cristóvão Park.

Source: the Author, 2021.

The results shown in Table 6 indicated that PSC, NH and CAJ extracts have the highest concentrations (mg/100g). The concentrations (mg/100g) are somehow related to the locational environmental stress that the plants suffered, which promoted a greater production of secondary metabolites (BORGES et al., 2017). The PSC, NH and CAJ samples were collected close to places with intense traffic, which could have directly contributed to the higher amount of polyphenols per sample. According to DE LIMA et.al., (2020), phenolic acids have antibacterial activity, so it can be inferred that their presence can contribute to making the extract a bactericidal agent. This approach will be better explored in topic: 4.2.3.

3 .1.4 High Performance Liquid Chromatography (HPLC-UV)

The proposed chromatographic analysis was validated by the analytical method of DOS SANTOS et.al., 2017 from the ethanol extracts of the araçá leaf, coming from the 5 locations in the city of Salvador-Ba (Table 1). From the chromatographic data obtained (table 7), it is possible to observe that two of the phenolic acids that presented high concentration, statistically comparing with the other samples, were ellagic and gallic acids. Considering their relevance as an antioxidant and with reports in the literature with the aid in the prevention of diseases, their addition to bodies of water in a container of still water can become an excellent point of view from a nutritional point of view for animals. vertebrates and antibacterial action (VIZZOTTO, 2012).

As a result, the determination of the 12 listed analytes was compared. The concentrations (mg 100 g $^{-1}$) of phenolic acids in the extracts (Table 8) were: ellagic acid (14.38 – 139.61); gallic acid (20.30 - 152.91); syringic acid (4.78 – 67.34); caffeic acid (0.40 - 2.55); ferulic acid (0.55 - 6.99); sinapic acid (1.10 - 16.29); chlorogenic acid (0.226 - 1.754); p-coumaric acid (0.25 - 2.80).

Table 7. Results for phenolic acid concentrations (mg 100 g $^{-1}$) in the extracts analyzed by HPLC-UV.

Sample	EA	GA	ASI	CA	FA	SA	CLA	APC
PSC	61.67	79.19	34.40	< LD	1.39	1.10	< LD	0.64
NH	139.61	69.25	<ld	2.42	4.37	4.51	<LD	1.26
CAB	58.54	62.91	35.54	0.40	1.69	7.94	<ld	0.25
BRT	60.35	52.30	67.34	2.44	0.55	16.29	0.226	3.69
CAJ	14.38	62.80	4.78	2.55	6.99	6.07	1.75	2.80

EA: Ellagic acid; GA: Gallic acid; ASI: Syringic acid; CA: Caffeic acid; FA: ferulic acid; SA: Synapic acid; ACL: chlorogenic acid; APC: p-coumaric acid PSC: Parque São Cristóvão; NH: New Horizon; CAB: Cabula; BRT: Brotas; CAJ: Cajazeiras; < LD means value less than detection limit.

Comparing the individual results, the NH and PSC samples had the best results among all the samples for the concentration of ellagic acid and gallic acid. Regarding syringic acid, BRT and CAB samples had the highest concentrations, and in NH there was no detection of this analyte. With regard to caffeic acid, CAJ, BRT and NH showed similar concentrations, but without detection in the PSC sample. Analyzing ferulic acid, NH and CAJ had greater expressiveness. Regarding sinapic acid, CAB, BRT and CAJ had the highest concentration. The concentration of chlorogenic acid in PSC, NH and CAJ was below the LD Finally, in p-coumaric acid all showed low concentrations when compared with the other analytes, with emphasis on CAJ and BRT. In general, the NH and PSC and CAB samples showed a higher concentration of phenolic acids, which corroborates the spectrophotometric data in Table 6, taking into account the standard deviation of the samples.

The concentrations (mg 100 g $^{-1}$) of flavonoids in the extracts (Table 9) were: catechin (81.81 – 361.09); Rutin (27.33 – 188.13); quercetin (245.28 - 628.54); kaempferol (0.17 - 58.51). Quercetin was one of the most expressive flavonoids in relation to concentration. Its antibacterial and larvicidal action has already been reported in the literature, but because it is a compound with low solubility in water, techniques are needed to make this analyte accessible to the environment. Thus, understanding that quercetin has high solubility in ethanolic extracts, it is possible to infer, through the results of table 9, that its presence in the extract may have potentiated the larvicidal effect shown in the table and contributed to the antibacterial activity (table 11), proving that the extract with the strawberry leaf is an excellent alternative for the proposed objective (PESSOA, ARAÚJO and SOUTO, 2018).

Table 8. Results for flavonoid concentrations (mg 100 g $^{-1}$) in extracts analyzed by HPLC-UV.

Sample	CT	RT	EQ	KP
PSC	81.81	148.92	628.54	41.03
NH	361.09	101.32	531.84	53.76
CAB	86.84	188.13	245.28	58.51
BRT	149.53	72.89	342.98	0.17
CAJ	104.60	27.33	569.14	52.99

CT: Catechin; RT: Rutin; QE: Quercetin; KP: Kaempferol; PSC: São Cristóvão Park; NH: New Horizon; CAB: Cabula; BRT: Brotas; CAJ: Cajazeiras;
< LD means value less than detection limit.
Source: the Author, 2021.

Comparing the individual results, the NH and BRT samples had expressive catechin concentrations when compared with the other samples. Regarding rutin, PSC and NH had the highest concentrations, with CAJ having an expressive minimum concentration. With regard to quercetin, PSC, NH and CAJ had significant concentrations already evidenced, similarly to the total flavonoid

content (table 6). Finally, in the concentration of kaempferol, CAB, NH and CAJ obtained the highest values.

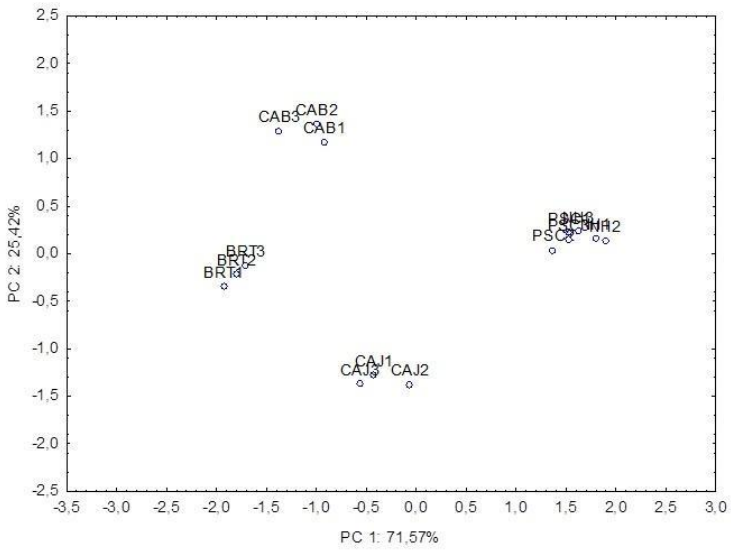
The concentrations (mg/100g), indicated in Tables 8 and 9, show that the locational environment interferes with the concentration of secondary metabolites in plants. Therefore, despite being of the same species, the relationship between the plant and the environment is closely related to the production, in this case, of phenolic acids and flavonoids, a fact that agrees with the literature (BORGES et al., 2017). In this sense, it is inferred that the extracts showed specific variations in the concentration of each analyte due to the plant's need for specific properties. Despite this difference in analyte concentration, the total phenolic acid and flavonoid content were close, without significant variations in values between spectrophotometry and chromatography, which corroborates the validation of the methods used.

3.1.5 Chemometric analyzes

3.1.5.1 Principal component analysis (PCA)

From the spectrophotometric analysis involving 5 samples (in triplicate) of the araçazeiro tree, the Principal Component Analysis (PCA) was performed, comprising 3 variables: total phenolic content (mg 100 g $^{-1}$), total flavonoid content (mg 100 g $^{-1}$) and saponin concentration (mg 100 g $^{-1}$). PCA analyzes were applied using the Statistica 7.0 software.

Figure 3. PC1 vs PC2 scores of the leaf samples of the guava tree (*Psidium guineense* Sw.)



BRT: Brotas; CAB: Cabula; CAJ: Cajazeiras; NH: New Horizon; PSC: São Cristóvão Park.

Source: the Author, 2021.

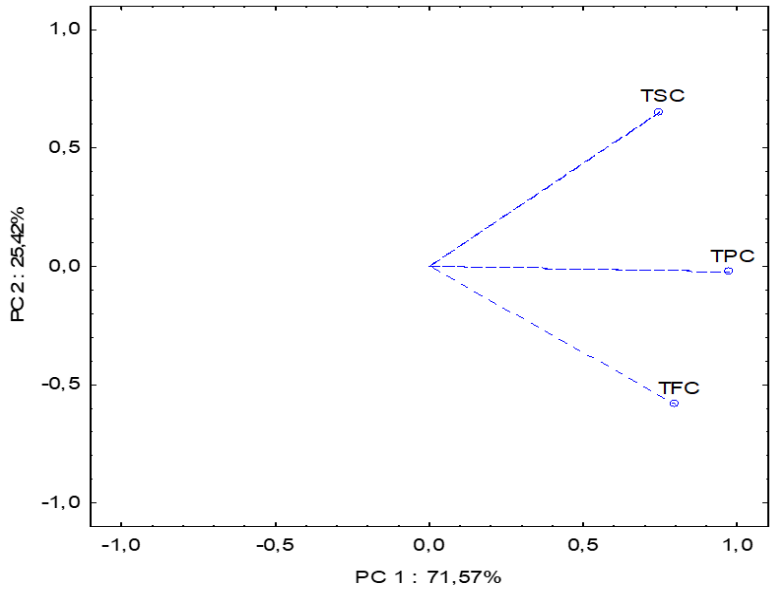
The components (PC1 × PC2) describe 96.99% of the total variance of the data and provide discriminatory information related to the samples, with the Principal Component (PC1) representing 71.57% of the total variance, and the second Principal Component (PC2) representing 25.42% of the variance.

From the analysis of the scores graph, it is possible to verify, through the grouping, that there is a relationship between the extracts of the PSC and NH samples, and that the extracts of the CAB, BRT and CAJ samples remained grouped only with their own group. This behavior can be justified due to the regions in which the samples were acquired, since according to BORGES et.al., (2017), the concentration of secondary metabolites in cultivars can occur in response to environmental stresses, such as the incidence of light, temperature , humidity, water, among others. Thus, it can be inferred that climate, soil, as well as contact with different types of pollutants, can significantly interfere with the concentrations of secondary metabolites present in plants. Therefore, as the PSC, NH and CAJ samples were acquired in roadside regions, where there is a constant supply of pollutants, it was already expected that they would be grouped in the same quadrant, however, the CAJ sample was presented in the lower quadrant isolated. Analyzing Table 5, it can be inferred that the reason why the proper sample was presented in a different quadrant was the concentrations of secondary metabolites, being lower in the PSC and NH samples.

Still in relation to figure 3, the extracts of the BRT and CAB samples were isolated in the same quadrant, which suggests that there is a similarity between them. This similarity can also be due to the regions in which the samples were acquired, which are regions where the flow of cars is not constant (private land), corroborating the premise that the conditions to which the plants are subjected can directly interfere with their growth. chemical composition.

Analyzing the Loading, indicated in figure 4, comparing with the scores chart (figure 3), it is possible to confirm, as evidenced in tables 4 and 6, a strong relationship between the content of total saponins and total phenolics and the samples PSC, NH and CAB, with the result of these groups being the highest compared to the other samples.

Figure 4. Loading PC1 vs PC2 of leaf samples from araçazeiro tree (*Psidium guineense* sw .)

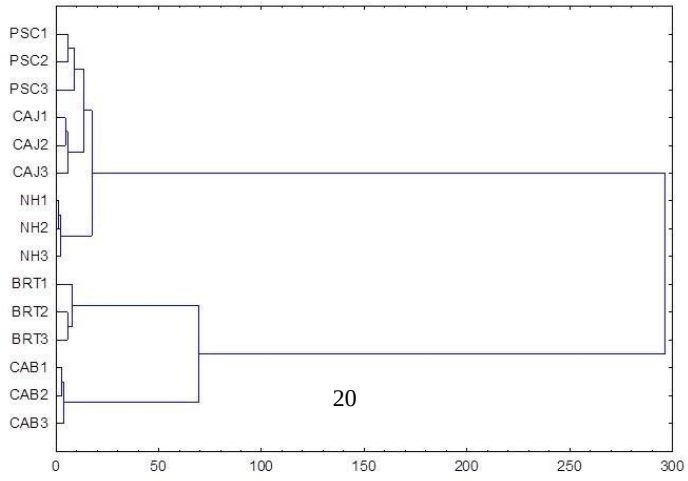


TSC: Total saponin content; TPC: Total phenolic content; TFC: Total flavonoid content.

3.1.5.2 Hierarchical Clustering Analysis (HCA)

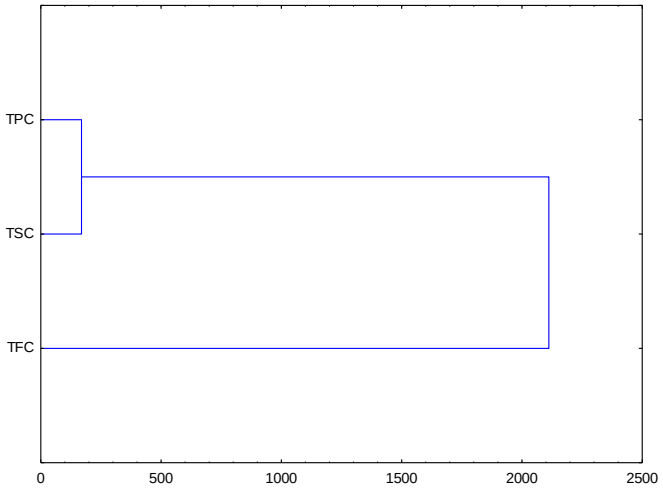
From the Hierarchical Cluster Analysis (HCA), the conclusion obtained in the principal components analysis (PCA) is evidenced more effectively, establishing the standards between the samples and the analyzed variables, indicated by figures 5 and 6.

Figure 5. Dendrogram of BRT, CAB, CAJ, NH and PSC samples by HCA.



BRT: Brotas; CAB: Kabula; CAJ: Cajazeiras; NH: New Horizon; PSC: São Cristóvão Park.
Source: the Author, 2021.

Figure 6. Dendrogram of TSC, TPC and TFC samples by HCA.



TSC: Total saponin content; TPC: Total phenolic content; TFC: Total flavonoid content
Source: the Author, 2021.

The information contained in figures 5 and 6 confirm the strong relationship between the PSC, CAJ and NH samples, as well as the relationship between BRT and CAB. The extracts from the PSC, CAJ and NH samples were grouped due to the concentration of TPC, while the extracts from the BRT and CAB samples were grouped due to the correlation of the concentration of TPC and TFC, corroborating the results of the PCA shown in figures 3 and 4. In contrast, all extracts from the samples were grouped by the high concentration of TFC in relation to the TPC and TSC contained in them. The results in figure 6 demonstrated that TSC interacted with TPC, because of its lower concentrations compared to TFC. This correlation corroborates the data in tables 4, 5 and 6 respectively.

The dendograms presented in figures 5 and 6 showed the correlation between all sample extracts through TFC. According to CROTEAU et al. (2000), the secondary metabolism of plants is linked to plant defense and resistance, which increases the probability of a higher flavonoid content. By correlating the biotic and abiotic factors with the results of figures 5 and 6, it is possible to infer that they were the predominant factors for the variation in the concentration of these bioactives in the plants.

3 .2 Efficiency tests

3.2.1 Evaluation of surfactant activity

The presence of saponins, which are characterized by persistent foam formation after agitation, can be evidenced in the height of the foam column in the test glassware. The difference in height (Table 9) between the samples showed that the extract with the highest foaming was NH and PSC. By correlating the data in Table 4 and Table 9, we noticed that the samples with the highest concentration of saponin also formed a larger persistent foam column. From this analysis, one can infer direct relationships between the concentration of saponins and foam height, as already evidenced in the literature, as well as the link between saponins and surfactant activity.

Table 9. Mean foam height results.

Samples	Mean foam height (mm) + DPR
PSC	22.4 ± 0.76
NH	27.2 ± 0.82
BRT	2.6 ± 0.80
CAB	18.3 ± 0.65
CAJ	15.86 ± 0.35

BRT: Brotas; CAB: Kabula; CAJ: Cajazeiras; NH: New Horizon; PSC: São Cristóvão Park.
Source: the Author, 2021.

According to CASTEJON, (2011), the fact that saponins decrease the surface tension of water is due to its molecular structure with an amphiphilic character, which decreases the intermolecular interactions of water on the surface, this makes foam formation easier (a water/air suspension). Therefore, it can be inferred that the samples, especially the aqueous extracts from the PSC and NH locations, which showed amounts of saponins, as well as persistent foam formation, have the potential to reduce the surface tension of the water. According to BESERRA et.al., (2010), mosquito larvae use surface tension to maintain stability when obtaining oxygen, which is used by cells to obtain energy (ATP). Therefore, based on the analyzes carried out, it can be deduced that the aqueous extracts, which have saponins and surfactant action, disfavor the survival of the *Aedes aegypti larvae* , since the larvae use the surface tension of the water to obtain oxygen and , subsequently, energy.

3.2.2 Evaluation of larvicidal activity

The results indicated in Table 10 demonstrated that the aqueous extracts (saponin) from all locations had larvicidal activity. Regarding the dilutions used (332 Kg/m ^{3}; 249 Kg/m ^{3}; 166 Kg/m ^{3}; 83 Kg/m ^{3}), it was verified that the larvicidal potential occurred in the first and second highest concentration of extracts, being 332 Kg/m ^{3} and 249Kg/m ^{3} respectively. The other concentrations proved to be unfeasible for this purpose, since the mortality rate (%) after 24 hours was zero, demonstrating that these concentrations were not efficient to cause significant damage to the *Aedes aegypti larvae.*

Table 10. Results of the relationship between extract concentration and mortality of *Aedes aegypti larvae* .

Samples	Extract Concentration (Kg/m ^{3})	Mortality rate (%)
Control	---	---
PSC	332	100
PSC	249	100
PSC	166	0
PSC	83	0
NH	332	100
NH	246	100

NH	166	0
NH	83	0
BRT	332	100
BRT	249	100
BRT	166	0
BRT	83	0
CAB	332	100
CAB	249	100
CAB	166	0
CAB	83	0
CAJ	332	100
CAJ	249	100
CAJ	166	0
CAJ	83	0

BRT: Brotas; CAB: Cabula; CAJ: Cajazeiras; NH: New Horizon; PSC: São Cristóvão Park.
Source: the Author, 2021.

The extracts were effective at the two highest concentrations tested, and may be an important bioactive to control the proliferation of the *Aedes aegypti mosquito* . The mechanism of action of saponins, as a larvicidal agent, is still little researched, but it is known that saponin, with its amphiphilic character, is capable of forming complexes with steroids, proteins and membrane phospholipids. Saponin also directly or indirectly modifies the membrane permeability of exposed cells. Therefore, it can be inferred that saponins, in agreement with the literature, may have acted on larval cells, which do not have significant protein protection, leading the larvae to death. (ANDRADE et.al., 2021; CASTJON, 2011).

3.2.3 Evaluation of antibacterial activity

The halo size results on the diffusion disks indicated that the extract has antibacterial activity. However, it presented variations in relation to the concentration and the different samples, as can be seen in Table 11. The PSC, NH and CAJ samples presented the largest halo sizes formed by the non-growth of the strain in the region, evidenced antibacterial activity. However, it was noticed that the best results were restricted to low concentrations, when related to the control. This behavior should be further studied, but it is suggested that plant

extracts in high concentrations may also serve as a nutritive environment, rather than just an inhibitory one.

Table 11. Results of the relationship between concentration and antibacterial potential by halo size.

Samples	Extract Concentration (Kg/m ^{3})	Halo size (mm)
Control	---	7.2
PSC	332	1.4
PSC	249	1.2
PSC	166	3.0
PSC	83	4.2
NH	332	1.0
NH	246	2.4
NH	166	3
NH	83	3.6
BRT	332	---
BRT	249	---
BRT	166	1.8
BRT	83	2.0
CAB	332	---
CAB	249	1.0
CAB	166	1.8
CAB	83	3.0
CAJ	332	---
CAJ	249	1.0
CAJ	166	2.2
CAJ	83	4.0

BRT: Brotas; CAB: Cabula; CAJ: Cajazeiras; NH: New Horizon; PSC: São Cristóvão Park.

Source: the Author, 2021.

Regarding the antibacterial activity, the extracts showed a significant potential when related to the control, especially the PSC, NH and CAJ samples (Table 11). These samples also had the highest concentrations of flavonoids and phenolic acids, as shown in tables 5 and 6. According to DE SOUZA ELLER et.al., 2015 and DE LIMA et.al., (2020), flavonoids and phenolic acids, belonging to the group of phenolic compounds, are the main antibacterial agents found in

plant extracts. Their action ranges from changing the permeability of the plasmatic membrane of simple cells to inhibiting important enzymes for protein synthesis. In the case of flavonoids, for example, this group of substances is capable of inhibiting bacterial topoisomerase, preventing the synthesis of DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). Therefore, it can be inferred that the amount of flavonoids and phenolic acids played a fundamental role in the antibacterial activity.

Another important point is the relationship that can be made with the *Aedes aegypti mosquito* and the antibacterial action. According to OSEI-POKU et al., (2012) and JUPATANAKUL et al., (2014), the bacteria in the environment contribute to the survival of mosquito larvae, because in this larval stage, especially in the L1 and L2 stages, the larva ingests these bacterial groups, which establish a symbiotic relationship in the gastrointestinal tract of these individuals. This relationship involves important biochemical exchanges between these populations, especially energy and biochemistry, which favor the survival and proliferation of larvae and mosquitoes, respectively. Therefore, it can be inferred that the death of bacterial organisms, in isolation (containers of still water without direct contact with the ecosystem), by plant extracts, which contain substances such as flavonoids and phenolic acids, can indirectly impact the survival of *Aedes aegypti larvae* and, consequently, reduce mosquito proliferation and their respective diseases.

3.2.4 Evaluation of extract toxicity

The results shown in Tables 12 and 13 showed that most of the extracts (aqueous and ethanolic) did not have, in 24 hours, relevant toxicity for microcrustaceans. Through the data obtained, it can be deduced that obtaining the extract did not result in a product with predominant acute toxicity, therefore, it can be inferred that the extracts have the potential to be applied in the ecosystem. More accurate tests are needed for complete toxicity analysis.

Table 12. Results of the relationship between concentration and mortality of *Artemia salina* (aqueous extract).

Samples	Extract Concentration (Kg/m ^{3})	Mortality (%)
Control	---	---
PSC	332	<50
PSC	249	<50
PSC	166	<50
PSC	83	<50
NH	332	<50
NH	249	<50
NH	166	<50
NH	83	<50
BRT	332	>50
BRT	249	>50
BRT	166	<50
BRT	83	<50
CAB	332	>50
CAB	249	>50
CAB	166	<50
CAB	83	<50
CAJ	332	<50
CAJ	249	<50
CAJ	166	<50
CAJ	83	<50

BRT: Brotas; CAB: Cabula; CAJ: Cajazeiras; NH: New Horizon; PSC: São Cristóvão Park.

Source: the Author, 2021.

It was possible to observe that the aqueous extracts from the BRT and CAB locations showed a potential risk, as shown in Table 12. These samples showed toxicity at high concentrations (332 Kg/m ^{3}; 249 Kg/m ^{3}), killing more than 50% of the population. population of *Artemia salina* after 24 hours of contact with the extract. Regarding PSC, NH and CAJ extracts, mortality did not exceed 50%, which demonstrates a potential applicable to the ecosystem.

Table 13. Results of the relationship between concentration and mortality of *Artemia salina* (ethanolic extract).

Samples	Extract Concentration (Kg/m ^{3})	Mortality (%)
Control	---	---
PSC	332	<50
PSC	249	<50
PSC	166	<50
PSC	83	<50
NH	332	<50
NH	249	<50
NH	166	<50
NH	83	<50
BRT	332	<50
BRT	249	<50
BRT	166	<50
BRT	83	<50
CAB	332	<50
CAB	249	<50
CAB	166	<50
CAB	83	<50
CAJ	332	<50
CAJ	249	<50
CAJ	166	<50
CAJ	83	<50

BRT: Brotas; CAB: Cabula; CAJ: Cajazeiras; NH: New Horizon; PSC: São Cristóvão Park.

Source: the Author, 2021.

The results in Table 13 demonstrate that the ethanolic extracts (phenolic acids and flavonoids) did not show acute toxicity related to any location and concentration. The *Artemia salina* remained alive and with standard movement after 24 hours. Therefore, the ethanol extract has a lower risk associated with its use, even at high concentrations, which demonstrates its potential for application to the ecosystem.

4. CONCLUSION

Plant extracts obtained from the leaves of the Araçazeiro (*Psidium guineense* Sw.) have the potential to serve as surface-active, larvicidal and antibacterial agents. The extracts from the locations: PSC, NH and CAJ proved

to be efficient in biochemical and applied tests, and may become an alternative method in relation to synthetic insecticides for controlling the proliferation of the *Aedes aegypti mosquito* and the diseases associated with these insects. In addition, the extracts from these locations presented a non-toxic and synergistic preliminary character to the environment, guaranteeing sustainability, health and accessibility. Through chemometric analyses, it was possible to observe that biotic and abiotic factors can significantly influence the content of secondary metabolites in plants , which represents an important point to be considered in the production of extracts. And finally, this work has as future perspectives to encapsulate the extract, as well as to better evaluate the toxicity of the extract (LD50 and in vertebrates) and to carry out tests of practical application in an isolated ecosystem.

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【評語】060014

1. This research used aqueous and ethanol extracts obtained from the leaves of the Aracazeiro collecting from 5 locations.
2. These extracts can be served as potential surface-active，larvicidal and antibacterial agents.
3. The author’s research theme is clear，the experimental design and analysis methods are scientifically appropriate，and have future application value.
4. Plant materials used in this study needs to be consistant.