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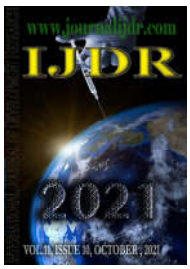

RESEARCH ARTICLE OPEN ACCESS

# CHEMICAL PROFILE AND LARVICIDAL ACTIVITY OF ANIBADUCKEI KOSTERMANS ESSENTIAL OIL

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**ABSTRACT**

The control of *Aedes aegypti* encounters numerous difficulties and the most relevant point is the resistance that the mosquito has been presenting to the insecticides used in its control. Among the products alternative to synthetic insecticides, essential oils can be highlighted. Therefore, this work aims to evaluate the action of the chemical composition and larvicidal activity against *Aedes aegypti* of the essential oil extracted from the bark of the stem of the species *Anibaduckei* Kostermans. The essential oil was extracted by the hydrodistillation technique in a Clevenger apparatus. The chemical characterization of the essential oil was performed by gas chromatography coupled to a mass spectrometer (GC-MS). Toxicity was measured by the artemia saline lethality bioassay. The larvicidal activity against *Aedes aegypti* was performed through the techniques recommended by the Ministry of Health. The essential oil presented THE LC50 of 9.89 mg L-1, being classified as active, and non-toxic by the toxicity assay. It presented linalool as the majority component, being reaffirmed by the literature used. Finally, this oil has substances that provide and encourage its application due to its potential for biological activities.



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# INTRODUCTION

Mosquitoes of the genus *Aedes* are the target in vector control, when it comes to measures to prevent arboviruses, even though it is a parameter of difficult success, since, for their control there are some requirements regarding the infrastructure of cities, which can be correlated with garbage collection, bursting in the water supply among others (ZARA *et al*., 2016). The most important arboviruses in Brazil, which circulate throughout the country in endemic regions, are Dengue (DEN) and Zika (ZIK) of the genus Flavivirus and Chikungunya (CHIK) of the genus Alphavirus (CAMPOS *et al*., 2018). In addition to being considered as a major public health problem, due to the clinical symptoms being similar, there is also a difficulty in differential diagnoses (CARVALHO *et al*., 2019). Considering that only yellow fever has an effective vaccine, according to the WHO the transmission of these arboviruses can be reduced with a control of the population of *Aedes aegypti*. However, the high degree of adaptation of this mosquito to the urban environment and the development of resistance to insecticides make it difficult to control its population density, increasing the cases of these diseases throughout Brazil (WHO, 2019). In Brazil, *Aedes Aegypti* control programs mainly use synthetic chemical insecticides, where temephos and pyroxyfen stand out. The control of *Aedes aegypti* encounters numerous difficulties and the most relevant point is the resistance that the mosquito has been presenting to these insecticides used in its control (DINIZ *et al*., 2014). Among the alternative products to synthetic insecticides, plants that present a complex metabolism can be highlighted, producing substances with various biological properties such as alkaloids, flavonoids, coumarins, saponins, terpenes, phenylpropanoids, among others. One of the most important products obtained from plants are essential oils, which are mixtures of secondary metabolites: monoterpenes, sesquiterpenes and phenylpropanoids, with volatile characteristic and which are produced by certain plant species with the main function of protection against microorganisms, herbivores, among others (LIMA, 2006; FREIRE, 2008). Essential oils have complex chemical composition and guarantee vegetables adaptive advantages in the environment in which they are inserted. They are used by plants in the same way they are used by humans - they fight infection, contain hormone-like

compounds, initiate cell regeneration and function as defenses against fungal, viral and animal enemies (MIRANDA *et al*., 2016). Thus, considering the problem of arboviruses and species diversity in our country, this work aims to evaluate the chemical composition and larvicidal activity against *Aedes aegypti* of the essential oil extracted from the bark of the stem species *Aniba duckei* Kostermans.

# MATERIALS AND METHODS

Samples of stem bark and thin branches were collected from three trees of *Aniba duckei* Kostermnas cultivated in the Ducke Forest Reserve, AM – 010, km 26, Manaus, Amazônas, Brazil (03º00"02" and 03º0800"'de latitude sul and 59º58' 00" west longitude). The essential oil of *Aniba duckei* Kostermnas was extracted by the hydrodistillation process in a Clevenger extractor system, coupled to a 6000mL round bottom balloon and, as a heat source, a heating blanket was used. In the extraction of the essential oil, approximately 30 grams of fine branches of the species *Aniba duckei* Kostermans with 300 mL of distilled water were weighed, maintaining the temperature of the heating blanket at 100 ºC. After 3 hours of distillation, the essential oil was collected. Subsequently, the oil was dried by percolation in Na2SO4 anhydrous. These operations were performed in triplicates and the samples were stored in glass jars under refrigeration, to avoid possible losses of volatile constituents. Then, these oils were submitted to the analyses. The extraction yield was calculated in the volume/mass and mass/mass ratio, observing the volume obtained in the extraction system itself. In the characterization of the physicochemical properties of the essential oil, the parameters of density, refraction index, solubility in ethanol (70% v/v), color and appearance of the oil were determined, using the methodologies of the Brazilian Pharmacopoeia (2019) for essential oils**.** The characterization of the chemical composition was performed by gas chromatography coupled to mass spectrometry (CG-MS) by the Usp Analytical Center. The essential oil was analyzed by gas chromatography (CG) in a Hewlett Packard device, model 5890, equipped with a capillary column SP 21000 dimethylpolysiloxane fused with silica, 30 m long, 0.25 mm in diameter and 0.1µm of film thickness. The column temperature program was 35º to 180ºC at 4ºC/min and 180º c to 280ºC at 20ºC/min. The drag gas used was helium gas (He), with a flow rate of 1 mL per minute. The samples were injected with a volume of 1µL. The oil components were identified in the same gas chromatograph, under the same conditions, but coupled to a mass spectrometer (CG-MS) HP 5971, operating at 70 eV. The relative percentage of components was calculated using the peak area of each substance on the chromatogram. Each substance was identified by comparing its mass spectrum with the spectra in the Wiley database. Antioxidant activity was performed by the spectrophotometric method of elimination of hydroxyl radicals from salicylic acid according to the methods described by Smirnff&Cumbes (1989) and Sundarajan *et al*. (2016). The essential oil at different concentrations of 10-500 mg L-1 was dissolved in DMSO 0.2%. 1 mL of salicylic acid (9 mM), 1 mL of ferrous sulfate (9 mM) and 1 mL of hydrogen peroxide (9 mM) were added to these concentrations. Ascorbic acid was used as a positive pattern. The reaction mixture was incubated for 60 min at 37 ºC in a water bath; after incubation, the absorbance of the mixtures was measured at 510 nm using a UV/VIS spectrophotometer and the calculated CE50.

The methodology described by Meyer *et al* was used to evaluate the lethality of Artemia salina Leach. (1982). Initially, a saline solution was prepared in the stock of each essential oil and nanoemulsions at the concentration of 10,000 mg L-1 and 0.02 mg of Tween 80 (active tense). Aliquots of 5, 50 and 500 µL of this were transferred to test tubes and supplemented with saline solution previously prepared up to 5 mL, obtaining at the end concentrations of 10, 100 and 1000 mg L-1, respectively. All trials were performed in triplicates, where ten larvae in the nauplium phase were transferred to each of the test tubes, as shown in Figure 1. For white, 5 mL of saline solution was used for positive control K2Cr2O7 and for negative control 5 mL of a 4 mg L-1 solution of Tween 80. After 24 hours of exposure, the live larvae were counted, considering dead those that do not move during observation or with the slight agitation of the vial. The Lethal Concentration 50% (LC50) for each essential oils and nanoemulsion was calculated based on the Reed&Muench method (1938), with classification of toxicity by the Dolabela criterion (1997). For anti-inflammatory activity, the protein denaturation method was followed as described by Padmanabhan & Jangles (2012).

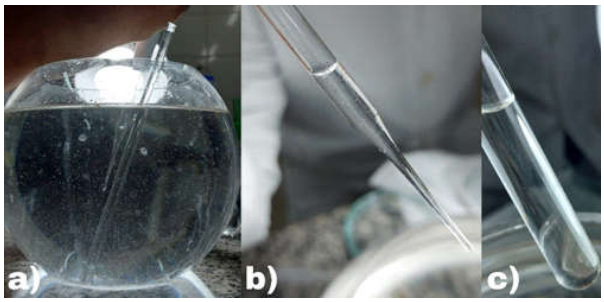


**Figure 1. Toxicity test with *Artemia salina* Leach. a)*Artemia salina* Leach culture aquarium b)Pasteur pipette to collect larvae c)test tube to check the lethality of *Artemia salina* Leach**

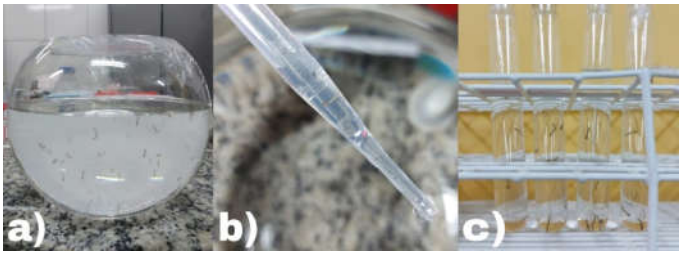


**Figure 2. Evaluation test of larvicidal activity of *Aniba duckei* Kostermans essential oil a) *Aedes aegypti* larvae culture aquarium b) dropper to collect stage 3 and 4 larvae c) test tubes to verify the larvicidal activity of *Aniba duckei* Kostermans essential oil**

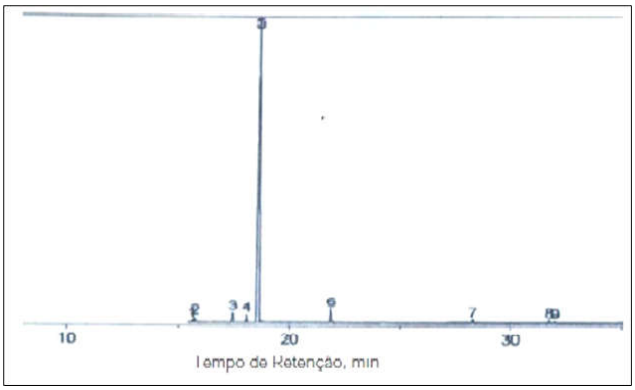


**Figura 3. Chromatogram of essential oil extracted from the branches of the species *Aniba duckei* Kostermans**

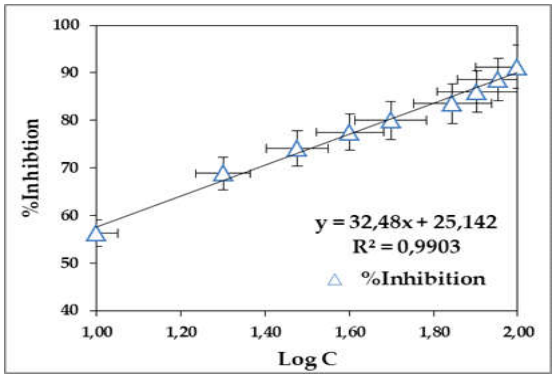


**Figure 4. Logarithm of concentration versus the percentage of inhibition for essential oil action by the hydroxyl radical discoloration method**

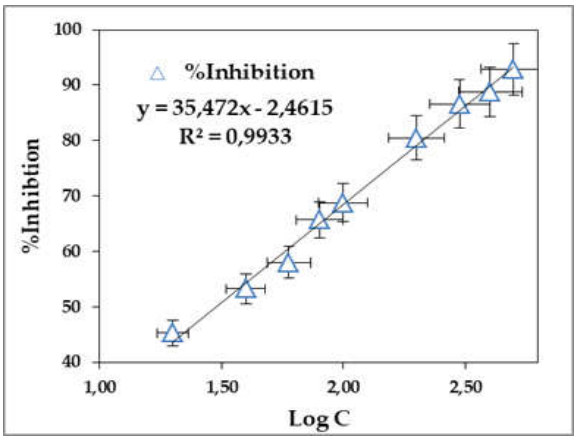


**Figura 5 Logarithm of concentration versus the percentage of inhibition for essential oil action by the protein denaturation method of albuminObserva-se na Figura 5 a equação da reta e o coeficiente linear para o cálculo da concentração eficiente 50%, conforme a Tabela 3**

**Table 1. Composition of the essencial oil of plant species *Aniba duckei* Kostrmans**

| Peak | RT (min) | Compound name | %Content |
|---|---|---|---|
| 1 | 15,61 | Limoneno | 0,52 |
| 2 | 15,71 | 1,8-cineol | 1,07 |
| 3 | 17,43 | Cis-óxido de linanol | 1,94 |
| 4 | 18,06 | Trans-óxido de linalol | 1,86 |
| 5 | 18,60 | Linalol | 89,34 |
| 6 | 21,88 | á-Terpineol | 3,06 |
| 7 | 28,26 | á-copaeno | 0,89 |
| 8 | 31,74 | á-Patchuleno | 0,77 |
| 9 | 32,02 | Cariofileno | 0,55 |

*RT (min) = Peak retention time by column elution order.*

**Table 2. Efficient Concentration 50% for the action of the essential oil *Aniba duckei* Kostermans**

| essential oil | $CE_{50}$ (µg $mL^{-1}$) | Equação da reta | $R^2$ |
|---|---|---|---|
| *Aniba duckei* Kostermans | 5,83 | y=32,48x+25,142 | 0,9903 |

**Table 3. Efficient Concentration 50% for the action of the essential oil *Aniba duckei* Kostermans**

| Extrato | $CE_{50}$ (µg $mL^{-1}$) | Equação da reta | $R^2$ |
|---|---|---|---|
| *Aniba duckei* Kostermans | 30,13 | y=35,472x+2,4615 | 0,9933 |

**Table 4. Efficient concentration 50% for larvicidal action of essential oil *Aniba duckei* Kostermans against *Aedes aegypti***

| *Concentration (mg $L^{-1}$)* | *Mortality (%)* | *$LC_{50}$ (mg $L^{-1}$)* | *σ* | *$\chi^2$* | *$R^2$* |
|---|---|---|---|---|---|
| *150* | *100,0* | *9,89* | *0,350* | *0,767* | *0,981* |
| *100* | *100,0* | *(7,22-13,54)* | | | |
| *80* | *100,0* | | | | |
| *60* | *100,0* | | | | |
| *40* | *100,0* | | | | |
| *20* | *100,0* | | | | |
| *15* | *70,0* | | | | |
| *10* | *50,0* | | | | |
| *5* | *20,0* | | | | |

Initially, the mother solution 500 mg L-1 was prepared in dimethylsulxide (DMSO 0.2%). Serial dilutions were performed in the concentration range of 10-400 mg L-1. The reaction mixture consisted of 2 mL of 10% albumin (PBS, pH=6.4) and 2 mL of the different concentrations of essential oil in test tubes, and were subsequently incubated in an oven at 37±1 °C for 15 minutes. The denaturation of the reaction compound was induced in a water bath at 60°C for 10 minutes.

After cooling, absorbance was measured in a UV-VIS spectrophotometer at a wavelength of 660 nm. Inhibition of protein denaturation was expressed as a percentage and The Efficient Concentration 50% (CE50/IC50) capable of inhibiting 50% of denaturation was expressed in mg L-1. The eggs were collected in São Luís/ MA, through traps called ovitrampas. These consist of brown buckets (500 mL), polyethylene, with 1 mL of beer yeast and 300 mL of running water and inserted two eucatex reeds for mosquito egg position. The traps were inspected weekly for the replacement of reeds and egg collection and sent to the Laboratory of Research and Application of Essential Oils (LOEPAV/UFMA) of the Technological Pavilion of the Federal University of Maranhão - UFMA.

Initially, the eggs of *Aedes aegypti* were placed to hatch at room temperature in a circular glass aquarium containing mineral water. The identification of the species followed the methodology proposed by Forattini (1962). The larvae obtained are fed with cat feed according to Silva's methodology (1995) until they reach the third and fourth stage, the age at which the experiments were carried out. The tests for larvicidal activity were carried out according to the adapted methodology proposed by Silva (2006). Initially, a mother solution of 100 mg L-1 of each of the essential oils was prepared and diluted in 2% DMSO solution and nanoemulsions (dilution-free). From this solution, five dilutions were prepared at concentrations 1.0-90.0 mg L-1. At each concentration, 10 larvae were added in the proportion 1 mL per larva, according to Figure 2. All tests were performed in triplicates and as negative control was used a solution formed of DMSO 2%, and as positive control, an ethanol solution (P.A) 70% v/v. After 24 h, the live and dead were counted, being considered dead, the larvae that do not react to the touch after 24 hours of the beginning of the experiment. To quantify the efficiency of essential oils and nanoemulsions, the statistical Test of Probit (Finney, 1952) and classification of the action by Cheng *et al* was applied. (2003).

# RESULTS AND DISCUSSION

The species *Aniba duckei* Kostermans, supplied an essential oil whose yield was 1.93% (m/m), which was considered of good value in relation to the extraction of other essential oils from aromatic plants. Oil density was 0.86 g mL-1. The solubility in ethanol at 70% (v/v) was in the proportion of 1:2. The refraction index (nD°25) was 1.46. The color observed was yellow, of clear appearance. There was an agreement with the values recorded by Azeredo (1958), with density of 0.87 to 0.89 g mL-1, refraction index of 1.46 to 1.47 and solubility in 2 volumes of alcoholic solution 70%. Teles (2003) found 0.86 g mL-1 for density, 1.46 for refraction index and solubility in ethanol 70% in the proportion of 1:2. Comparing the values for the essential oil of the branches of the species *Aniba duckei* Kostermas with those of the literature, the results found were similar. Figure 3 shows the chromatogram of the essential oil extracted from the branches of the species *Aniba duckei* Kostermans. Table 1 presents the chemical composition of the essential oil of *Aniba duckei* Kostermans. Table 1 shows Linalool as the majority compound. This result is in accordance with those found by Teles *et al*. (2017) when analyzing by CG/MS the essential oil of the species under study. Similar results were also observed by Araújo *et al*. (1971) when analyzing the essential oil of the leaves and branches of an individual of *Aniba duckei* Kostermans of the Ducke Reserve, verifying that the linalol content varies according to the seasonality of the collection. Also, in wood, linalool contents from 80 to 92% can be found, as reported in the literature (Alcântara *et al*. 2010). Figure 4 presents data referring to the natural logarithm of the concentration versus the percentage of discoloration of hydroxyl radicals for the action of *Aniba duckei* essential oil. Figure 4 shows the equation of the line and the linear coefficient for the calculation of the efficient concentration 50%, according to Table 2. According to Table 2, the analyzed essential oil is classified as active, according to the criteria of Campos *et al*. (2003). It was not possible to find in the literature results of studies on the antioxidant activity of the essential oil of *Aniba duckei* Kostermans obtained by the methods described by Smirnff&Cumbes (1989) and Sundarajan *et al*. (2016).

According to Teles *et al*. (2021), the EC found was 15.46 µg mL-1 for essential oil extracted from the same plant species and 6.78 µg mL-1 for Linalool by the ABTS method, results that were also classified as active, but the antioxidant capacity observed in this study was more efficient than that reported by the authors. Ferreira *et al*. (2021) found the EC of 40.06 mg L-1 by the DPPH method and 48.67 mg L-1 by the ABTS method. These results are also lower than that observed in this study, emphasizing the potential criterion of activity that the essential oil analyzed presented in this trial. Figure 5 presents data referring to the natural logarithm of the concentration versus the percentage of inhibition of protein denaturation for the action of *Aniba duckei* essential oil. Table 3 shows that the essential oil of A. duckei has anti-inflammatory activity. Queiroga *et al*. (2006) also found that the essential oil of the plant species presents anti-inflammatory activity, being used by local populations of the Amazon for the treatment of rheumatic diseases and other natures. As described by Peana *et al*., (2003 and 2004a), the administration of linalool (majority compound) induces antinociceptive and anti-inflammatory effect in different experimental models. According to the results obtained, it was possible to obtain the intersection of curves at 2.45 and CL50 at 282 mg L-1 ± 2.95 mg L-1 and according to Dolabela (1997) is classified as non-toxic. Studies in the literature on toxicity by the Artemia salina Leach bioassay in front of The OE of A. duckei are still scarce and little publicized. Therefore, the results related to toxicity were compared to studies that present linalool as the major component.

The chemical composition correlates the major compound linalool as nontoxic being used in the medical area, justifying the result found in its classification. Fujiwara *et al*. (2017) verified linalool toxicity by artemia saline preliminary toxicity bioassay obtaining CL50 275.2 µg mL-1 by classifying linalool compound as nontoxic. Goel *et al*. (2019) state that linalool is nontoxic, thus confirming applicability as a tool for manipulation in cancer cells, because it has a cytostatic effect (Rodenak-Kladiniew *et al*., 2018). Thus it can be stated that nontoxic OE's can also have a relative efficiency in larvicide properties.A Tabela 4 apresenta os dados referentes a mortalidade das larvas *Aedes aegypti* para ação do óleo essencial de *Aniba duckei*. Table 4 shows that we can classify highly active essential oil according to Dias&Moraes (2014). The authors establish that the larvicidal potential is classified according to the criteria based on lethal concentration (LC), where the OE's that obtain CL50>100 mg L-1, are considered non-active, those who obtain LC50 <100 mg L-1 are considered active and those who obtain CL50<50mg L-1 are highly active. Thus, the OE of *Aniba duckei* Kostermans showed highly efficient larvicidal action, by keeping the LC50 below 50 mg L-1, encouraging its potential and use. According to Teles *et al*. (2017) when analyzing the larvicidal activity of the OE of *Aniba duckei* Kostermans, for linalool patterns, the main component of *Aniba duckei* Kostermans essential oil, l-linalool killed 100% of the larvae at lower concentrations of 350 µg mL-1, where in vitro essential oil reached only 100% to 400µg mL-1 and d-linalool did not reach this level in the concentration range analyzed. Thus, they concluded that linalool responsible for larvicidal activity should be l-linalool.

# CONCLUSION

Through the results obtained it was possible to identify linalool as the majority compound, responsible for the satisfactory results on the anti-inflammatory capacity of *Aniba duckei* Kostermans essential oil and also its antioxidant activity, besides presenting highly efficient larvicidal action. It was also concluded its classification as non-toxic essential oil, so we can affirm that this oil has substances that provide and encourage its application due to its potential for biological activities.

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