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RESEARCH ARTICLE

Chemical Characterization and Larvicidal Activity of Essential Oil from *Aniba duckei* Kostermans against *Aedes aegypti*

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ABSTRACT- *Aedes aegypti* mosquito is the major vector of zika, chikungunya, and dengue fever. These diseases incidence has been growing rapidly in many points of the globe in the past few years. And because there's no vaccine for them yet, the best way to fight those diseases is to attack their vector, specially by eliminating potential sites for its oviposition and larvae growth. Nowadays, organophosphorus insecticides are used in increasing doses, which targets *A. aegypti* resistant populations. *Aniba duckei* Kostermans, which is known as rosewood and belongs to the Lauraceae family, is a species with trees up to 30 meters tall and 1 meter in trunk diameter. Its essential oil is used in perfumery due to its high content of linalool. This research identified the components of essential oil from *A. duckei* Kostermans thin branches and leaves and then applied it as larvicide against *A. aegypti*, and its effects were measured by calculation of concentration at which half larvae die (LC_{50}). Average yield found in oil from the plant was 1.93% by mass. The major component in rosewood essential oil is linalool, whose concentration was found 89.34% by mass. LC_{50} for the essential oil was 250.61 (±2.20) µg mL^{-1}, for l-linalool, 279.89 (±2.12) µg mL^{-1}, and for dl-linalool was 346.73 (±2.14) µg mL^{-1}.

Key-words- Essential oil, *Aniba duckei* Kostermans, Linalool, *Aedes aegypti*, Larvicide

INTRODUCTION

The world has experienced a dengue incidence increase in the last 50 years. Recent studies estimate about 395 million cases of dengue hemorrhagic fever in 100 countries, of which 500 thousand are classified as dengue hemorrhagic fever/ dengue shock syndrome (DHF / DSS) [1]. Disease is caused by four serotypes of dengue virus, DENV-1, DENV-2, DENV-3 and DENV-4 [2].

This is the most important arbovirosis worldwide with about 50 million infections per year [3], and it can be asymptomatic or manifest many symptoms, from self-limited febrile illness to severe forms that may lead to death [4].

In terms of morbidity and mortality, dengue is nowadays considered the most important viral disease transmitted by mosquitoes, constituting a serious public health problem of urban centers from South & Central America, Southeast Asia and West Pacific tropical areas [5]. Chikungunya disease, which shown symptoms similar to dengue's is caused by Chikungunya virus (CHIKV), an RNA virus member of the *Alphavirus* genus in the family *Togaviridae*, first described in Africa, but which migrated later to Asia and Europe, after small mutations [6-8]. These disease symptoms, which may persist for months or even years, are debilitating, causing fever, arthralgia or severe arthritis and itchy skin [9].

Zika virus is a flavivirus (*Flaviviridae* family) originally isolated in Uganda, in 1947 [10]. From 1951 to 2013, serological evidence in humans were notified in African countries (Uganda, Tanzania, Egypt, Central African Republic, Sierra Leone and Gabon), Asian countries (India, Malaysia, Philippines, Thailand, Vietnam and Indonesia) and Oceanian countries (Micronesia and French Polynesia). In the Americas, zika virus was identified in Easter Island, Chile's territory in the Pacific Ocean, which was 3.500 km from the mainland, only in the beginning of 2014 [11].

Since May 2015, Brazil's Ministry of Health has been registering cases of zika virus in the country [12]. Usually, infection is characterized by fever, skin rash, joint pain or conjunctivitis, that may last for days or weeks, and its

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symptoms are many times confused with dengue's or chikungunya's, which may result in diagnostic errors [13].

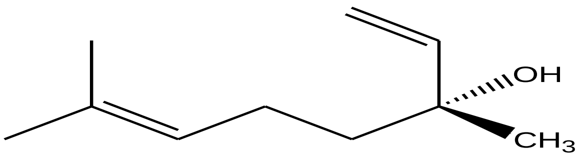
Dengue, chikungunya and zika are all transmitted by the same vector, *A. aegypti* mosquito [8,10,14-15]. Because there are still no validated vaccines against dengue or a specific antiviral for treatment of those diseases [16-18], the best control method is prevention, by attacking its vector [19]. Vector control is done by eliminating propitious locations for oviposition or by fighting these mosquito larvae. In recent times, this combat has been carried out by applications of organophosphorus insecticides in increasing doses, which has caused mosquitoes to become resistant to pesticides [20,21].

Plants that are source of molecules with phage inhibitory, repellent and insecticidal actions, in addition to substances that are able to change growth regulation, are a good alternative to the use of insecticides. Essential oils, produced in the secondary metabolism of plants, have also been shown to be a good source of materials with insecticidal, larvicidal and repellent action [15,22-25].

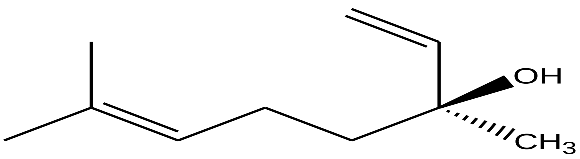
Botanical species *A. duckei* Kostermans, of Lauraceae family [26,27], synonym of *A. rosaeodora* Ducke [28-30], has many common names, like: pau-rosa, pau-rosa-do-amazonas and umbauba (Brazil), rosewood (English speaking countries), bois de rose femelle (French Guyana), enclit rosenhout (Suriname), cara-cara (Guyana) [30] and palo de rosa, in Castilian speaking Amazonian countries [31].

Linalool (3,7-Dimethyl-1,6-octadien-3-ol), shown in Fig. 1 is the major component of *A. duckei* Kostermans essential oil [32]. Other minor components are also present in this essential oil's composition.

Linalool, which is an alcoholic monoterpene and one of the most important substances for the fragrance industry [33], occurs naturally as two stereoisomers, 3R-(-)-linalool and 3S-(+)-linalool [34]. Fig. 1 (A and B) below has shown the structures for linalool.



(A)



(B)

Fig. 1: Enantiomeric structures for linalool:
(A) 3R-(-)-linalool or lincareol;
(B) 3S-(+)-linalool or coryandrol

To contribute in the fight against *A. aegypti* larvae, the essential oil from *A. duckei* Kostermans was extracted, and then its physical-chemical properties were evaluated, as well as its larvicidal activity against larvae of the *A. aegypti* mosquito in third or fourth stages.

MATERIALS AND METHODS

This research was developed in the Laboratory of Fuels and Materials (LACOM) located at Paraiba Federal University (UFPB) in partnership with Laboratory of Analytical Chemistry (LPQA), Analytical Center and Laboratory of Physical Chemistry, Microbiology of the Technological Pavilion of the Federal University of Maranhao (UFMA), Laboratory of Researches and Tests of Fuels (LAPEC) of the Federal University of Amazonas (UFAM) and Sao Carlos Institute of Chemistry from University of Sao Paulo (USP).

Samples, leaves and thin branches, collected from three *A. duckei* Kostermans trees cultivated in the Ducke Forest Reserve, highway AM–010, 26 km, Manaus, Amazon, Brazil (03º00"02" and 03º08"00" south latitude and 59º58'00" west longitude), were dried for seven days under natural ventilation and then crushed. Essential oil was extracted from 30 grams of thin branches with 300 mL of distilled water, by hydro distillation using Clevenger system, under the temperature of 100°C. After that, the oil was dried by percolation with anhydrous Na_2SO_4 and then stored in glass ampoules under refrigeration.

Yield, density, extraction time, ethanol solubility, refractive index, oil extraction yield, color and appearance were determined. As standards were used racemic linalool from Aldrich (Aldrich Chemical Co) and R-(-)-linalool from Fluka (Fluka Chemie GmbH). Standard solutions of monoterpenes in ethanol and in hexane were prepared by dilution at different concentrations.

GC-MS essential oil analysis was performed on a Varian chromatograph, model 3900, using helium as carrier gas with the flow in the column of 1 mL min-^{1}; Injector temperature: 270°C, split 1:50; capillary column (30 mx 25 mm) by stationary phase VF-1ms (100% methylsiloxane 0.25 μm) and oven temperature programmed to 60°C and then increased to 220°C at a rate of 4°C min-1 and then increased again to 260°C, this time at a rate of 1°C min-^{1}, with total running time of 100 minutes. For mass spectrometer, the manifold, ion trap and transfer line temperatures were set to 50, 190 and 200°C, respectively. 1.0 μL (automatic injector CP-8410) aliquots of the samples diluted were injected in the proportion of 20 μL for 1.5 mL of hexane. Linalool was quantified by the external standard method, considering its high concentration in the samples.

In order to collect *A. aegypti* eggs, a simple trap was prepared using 500 ml plastic jars half-filled with water and a piece of wood of approximately 20 cm x 5 cm with one part submerged. For hatching, the eggs were immersed in a plastic container with 3 liters of mineral water and 500 milligrams of rat feed. After immersion of the eggs, 0.5 g more of rat feed was added, to aid in larvae growth. All material was kept inside a wooden cage and was covered with a fabric screen, suitable for insects, in order to avoid contamination of eggs of other mosquitoes' species. After hatching, the larvae were monitored until they reached the 3rd or 4th stage of development, from 4 to 5 days, when they were then used in the larvicidal activity tests.

For toxicity test, 10 larvae were transferred to a beaker containing 20 mL of mineral water (26–28°C). Each test was carried out five times for each concentration tested. Positive controls were performed with the organophosphate temephos in *A. aegypti* larvae at the concentration used by the sanitary surveillance which is 100 ppm. Negative controls were performed with 20 mL mineral water (26–28°C) containing 0.04% Tween. Larvae were exposed to the solutions for 24 hours and at the end of this period mortality was recorded.

STATISTICAL ANALYSIS

Statistical analysis of data was performed according to the Reed-Muench method of plotting the mortality data for each concentration tested, where one curve is observed for accumulation of dead animals at each concentration and another one for accumulation of survivors. The point of intersection between the curves is the median lethal concentration (LC_{50}), because at this point the number of surviving animals is equal to the number of dead animals [35]. Confidence interval was calculated according to the PIZZI method [36].

RESULTS AND DISCUSSION

The extraction time with the best yield was obtained after four hours of extraction, yielding 1.87% (m/m). Density, 70% (v/v) ethanol solubility, and refractive index for this essential oil were respectively 0.86 g/mL, 1:2 and 1.46. These data, together with the yellow color and clear appearance observed, are in agreement with literature data [37].

The substances identified from the chromatogram are listed in Table 1. For identification of the compounds were used the spectral databases of the spectral libraries NIST105, NIST21 and WILEY139, and AMSDIS (Automated Mass Spectral De-convolution Mass & Identification System) software, as well as references [38]. For linalool, confirmation was also by the addition of standard.

Table 1: Identified compounds in a sample of essential oil from *A. duckei* Kostermans' branches

Pico	t_{RET}^a	Compound Name	%A^b
1	15.61	Limonene	0.52
2	15.71	1,8-Cineole	1.07
3	17.43	Cis-linalool oxide	1.94
4	18.06	Trans-linalool oxide	1.86
5	18.60	Linalool	89.34
6	21.88	α-Terpineol	3.06
7	28.26	α-Copaene	0.89
8	31.74	α-Patchoulene	0.77
9	32.02	Caryophyllene	0.55

a= Peak retention time by column elution order
%A^b = normalized area percentage

From the graph it's possible to see linalool, $C_{10}H_{18}O$, as the major component, with 89.34%, followed by α-terpineol, $C_{10}H_{18}O$, whose area percentage was 3.06%.

The larvicidal activity of essential oil from *A. duckei* Kostermans was tested in seven concentrations: 100, 150, 200, 250, 300, 350 and 400 µg mL^{-1}, with 10 larvae used for each concentration. The tests were performed five times for each concentration and data on the number of live and dead larvae was obtained from an average of the five replicates.

For linalool (dl-linalool and l-linalool) standards, major component of the essential oil from *A. duckei* Kostermans, larvicidal activity was tested at the same seven concentrations at which the essential oil was tested, also five times for each concentration. The results are summarized in Table 2.

Table 2: Estimation of LC50 of essential oil, and linalool (dl-linalool and l-linalool) by Reed-Muench method based on accumulation of dead and live larvae

Doses (µg mL^{-1})	Log dose	Mortality (%)		
		Oil	dl-linalool	l-linalool
400	2.60	100	66.0	100
350	2.54	76	38.7	100
300	2.48	56	28.0	44
250	2.40	40	16.0	34
200	2.30	34	6.0	18
150	2.18	30	0.0	4
100	2.0	18	0.0	0

L-linalool killed 100% of the larvae at lower concentrations, from 350 µg mL^{-1}, where the oil alone has only reached 100% at 400 µg mL^{-1} and dl-linalool has not reached this level in the analyzed concentration range. When investigating median lethal concentration (LC_{50}), the best larvicidal activity was detected for the essential oil from *A. duckei* Kostermans, LC_{50}=250.61 (±2.20) µg mL^{-1}, against LC_{50} of 279.89 (±2.12) µg mL^{-1} of l-linalool and LC_{50}=346.73 (±2.14) µg mL^{-1} for dl-linalool. Thus, it is concluded that the linalool responsible for larvicidal activity should be the levorotatory isomer (l-linalool). No information was found though, in the literature data, on larvicidal activity against *A. aegypti* for l-linalool, whereas for dl-linalool, the results obtained are in accordance with the literature data, which does not attribute to linalool a value of larvicidal activity, but to the interval greater than 100 µg L^{-1} (> 100 µg L^{-1}) [39].

CONCLUSIONS

In this research, essential oil from *A. duckei* Kostermans presented 1.87% (m/m) extraction yield, with linalool being its major component (89.34%), followed by α-terpineol (3.06%). The best result of median lethal concentration (LC_{50}) against *A. aegypti* was the one of the essential oil, followed by the results for l-linalool, which is responsible for linalool's larvicidal. Once essential oil is a natural product and, therefore, less harmful to humans' and domestic animals' health, it can be used as a larvicide in at larval growth sites by *A. aegypti* in order to reduce the impact of synthetic insecticides on the health of people and the environment. Besides, the complex composition of the essential oil makes it harder for mosquitoes to develop resistance. Other advantages of essential oil from *A. duckei* Kostermans discovered during this research includes environmental, economic and social aspects, since the oil is prepared using just leaves and thin branches from reforested plants, its final cost is low compared to synthetic insecticides' and it also can generate jobs, and income to local residents, from production to commerce.

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