Chemical characterization and larvicidal properties of the essential oils of against *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT

The mosquito of the genus *Aedes* is the arthropod responsible for transmission of the dengue virus. Some procedures are proposed to prevent the proliferation of this vector, including the use of larvicides and insecticides. The present work has as the extraction of essential oils from the species *Syzygium aromaticum* and *Ocimun grantissimun* and the evaluation of their active components against *Aedes aegypti* larvae. The oils were subjected to GC-MS analysis to identify the majoritarys compounds. The EOs were subjected to turbidimetric tests to obtain the degree of solubility in 1% DMSO, acetone, alcohol and Tween 80. The analysis of EOs by GC–MS resulted in the identification of eugenol, 1-8-cineole and β-caryophyllene. Tests with the enzyme acetylcholinesterase were performed following the protocol of Ellman adapted by Rhee. The turbidity of the EOs in ethanol presented values from 30 to 102 NTU, implying the presence of a greater number of soluble molecules. Studies of larvicidal assays of *S. aromaticum* and *O. gratissimun* oils showed LC_{50} values of 39.5 and 54.6 mg/ml respectively. For anticholinesterase activity, *O. gratissimun* presented an IC_{50} of 6.2 mg/ml. The present work suggests the essential oils under study are promising in the formulation of larvicides and insecticides.

Keywords: *Aedes aegypti*, *Ocimum gratissimum*, *Syzygium aromaticum*, larvicidal, essential oil.

INTRODUCTION

Aedes aegypti (Diptera: Culicidae) is characterized as a species extremely adapted to human interaction. Its presence is more common in urban areas, mainly in regions of high population density, in these spaces the female has more opportunities for feeding and there are more breeding sites places for spawning to occur. Measures are adopted to prevent the proliferation of this vector, among which preventive actions to eliminate breeding sites stand out, as the mosquito reproduces easily in containers containing water, and the increase in temperature, the ability to develop in waters with different degrees of pollution and the creation of new breeding sites are factors that favor the hatching of mosquito larvae (Azevedo 2015).

The larval stage is the period of feeding and growth of the insect, having four stages (L1, L2, L3 and L4). Temperature, food availability and density of larvae in the hatchery are factors that favor development, the low temperature around 20 °C and the scarcity of food can extend for several weeks, the transformation of the 4th stage larvae into pupae. From egg to adult, the life cycle can take a period of 7 to 10 days. Therefore, for the cycle to be interrupted, the elimination of breeding sites must be carried out at least once a week (Saude 2001).

Natural insecticides, especially those derived from plants, are the most promising, as they are ecologically effective and generally have

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low toxicity (Ansaria et al. 2000; Benelli et al. 2013; Santos et al. 2022). The WHO (World Health Organization) defines some important points in the preparation and evaluation of larvicidal activity, among which small and large-scale field trials, residual effects in different ecological contexts and comparative analysis with other insecticides stand out. Within this context, plants are promising for the production of larvicides and insecticides, so the present work aimed at the chemical characterization and larvicidal activity of essential oils extracted from the flowers of *Syzygium aromaticum* (L.) Merr. & L.M.Perry and leaves of *Ocimum gratissimum* L. against *Ae. aegypti*.

MATERIALS AND METHODS

Plant material – collection

Syzygium aromaticum flowers were purchased at the Central Market, located at Rua João Cabral, 549, Teresina, Brazil. (5°05'26.0" S 42°49'08.7" W). The collection of the leaves of *Ocimum gratissimum* was carried out in the municipality of Piracuruca, Brazil, under the coordinates: 3°55'09.5" S, 41°42'11.0" W, on June 1, 2018 at 11:30 am. A voucher with the number 31,927 was obtained from the herbarium Graziela Barroso at the Federal University of (UFPI) and identified by Prof. Dr. Roseli Farias Melo de Barros.

Extraction of Essential Oil (EO)

The leaves of *O. gratissimum* were dried in an oven at 40 °C for 48 h, according to the methodology proposed by Campos et al. (2014). After drying, the leaves were crushed, weighed and about 320 g of the leaves were submitted to a Clevenger-type hydrodistillation system for about 3 h. For the extraction of EO from *S. aromaticum*, about 1000 g of the flowers were submitted to hydrodistillation.

Gas Chromatography coupled to Mass Spectrometry

Chromatographic analyzes were performed on the SHIMADZU equipment, model GCMS-QP2010 SE, equipped with an SLB-5ms column. The heating schedule for the chromatographic furnace established was from 60 °C (0 min) → 3 °C/min to 246 °C (10 min) The instrumental parameters used were: injector temperature of 220 °C; 1:10 split injection mode; volumetric flow of the mobile phase (Helium) of 0.59 ml/min; interface temperature of 300 °C; analyzes performed in SCAN mode in a range of 47 to 400 *m/z* (in 0.5 s intervals and with 70 eV ionization energy); detector temperature of 250 °C.

Physical characteristics of OEs - Solubility

The degree of solubility of the samples in DMSO 1%, acetone, alcohol and Tween 80 were carried out through turbidimetric tests using a TB-2000 digital bench turbidimeter, with turbidity range (NTU) 0 - 10000, and solids concentration solutions. in suspension (CSS) of 100 and 200 mg/ml (100 and 200 mg of OE per liter of solvent - DMSO, Acetone, Ethanol and Tween 20).

Larvicidal bioassay

The larvicidal activity of essential oils was assessed using an adaptation of the method recommended by the World Health Organization/2005 (WHO/2005). The bioassay was carried out at the Laboratory of Parasitology and Sanitary Entomology (LAPES-UFPI).

Around 400 larvae of *Ae. aegypti* were obtained from adult mosquitoes, from Teresina-PI, kept in a semi-closed insectary at LAPES-UFPI, at 28 ± 1 °C and 70 ± 10% RH. The adults were fed with concentrated sugar solution and the females performed the blood meal in mice. The larvae were fed with crushed fish food during for 5 days.

The tests were performed for each concentration of the sample (0, 20, 40, 60, 80, and 100 mg/ml), and for each test a negative control was included with distilled water, containing the same amount of the best solubility solvent as in the test sample. One hundred and eighty larvae s of the *Ae. aegypti* mosquito in the initial fourth instar (L-4, white head), aged 3 to 5 days, were placed in disposable cups containing the solution. The number of dead larvae as well as morphological and behavioral changes was recorded after 24 h of the beginning of the experiment and again after a period of 48 h. Larvae were considered dead when they did not respond to stimuli or when they did not maintain a downward and upward movement to the solution surface. The LC_{50} for larvicidal bioassays performed in triplicate was calculated using Probit survival analysis, with a 95% confidence interval were calculated using the Microsoft® Excel program.

RESULTS AND DISCUSSION

Extraction of Essential Oil

Table 1 shows the yields of the essential oils of *S. aromaticum* (EOSA) and *O. gratissimum* (EOOG), which are calculated from the mass of plant varieties and the mass of the oil obtained.

Chromatographic analysis of the EOs

The analysis of the EOSA by GC-MS resulted in the identification of 6 substances,

representing more than 97% of the composition. The main constituents were: eugenol, β-caryophyllene and eugenyl acetate (Figure 1 and Table 2).

Sharma et al. (2017) identified 31 constituents, the most abundant being eugenol, which presented an absolute abundance of 75.41%. The other main components of the oil were β-caryophyllene (15.11%), α-humulene (3.78%), caryophyllene oxide (1.13%), δ-cardinene (0.84%) and alloaromadendrene with 0 .51%. Hossain et al. (2014) identified 21 chemical compounds, in which eugenol was present in 57.17% of the total oil composition and β-caryophyllene was present in 29.94%. The essential oil extracted from the seeds of *S. aromaticum* by Machado et al. (2011) was analyzed by GC and GC-MS, eugenol was identified as the main constituent, present in about 85.3% of the total composition of the essential oil. Eugenol

Table 1. The yield of essential oils obtained from flowers of *Syzygium aromaticum* and leaves of *Ocimum gratissimum*.

Plant Material	Initial mass (g)	OE mass (g)	Performance (%)
EOSA	1000	20	2.00
EOOG	320	5	1.56

The yield obtained in the present study is consistent with the yield obtained for the extraction of oils already reported in the literature, which varies from 0.3 to 3.6% on a dry basis (Morais 2006).

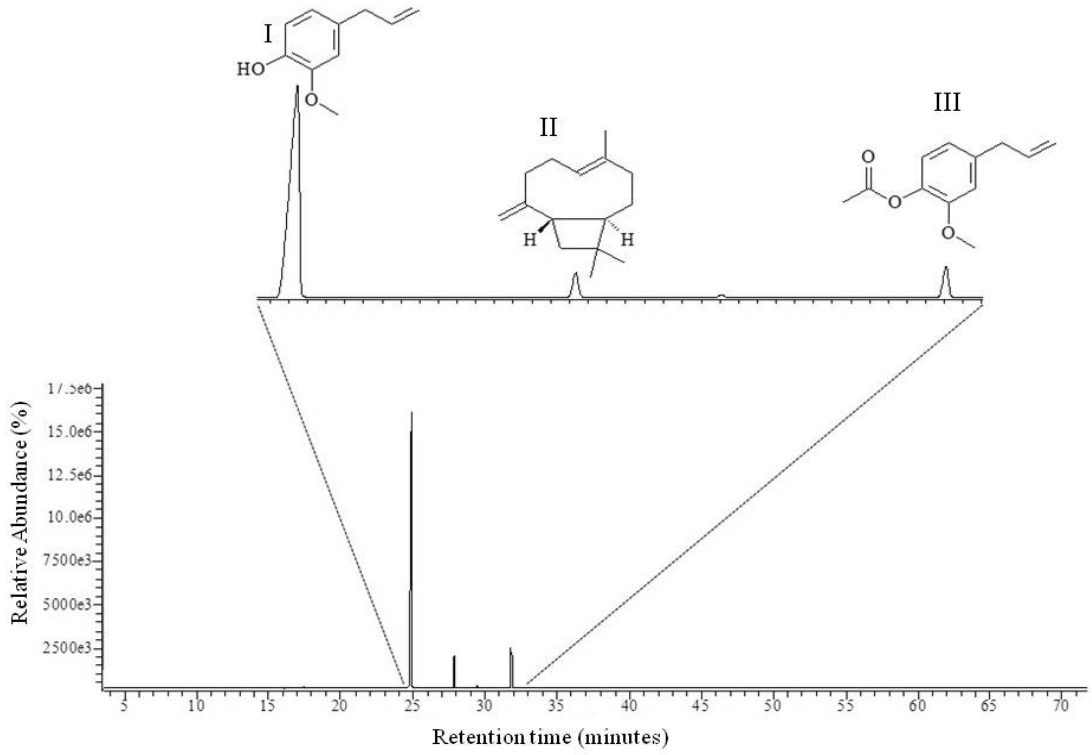

Figure 1. Chromatographic profile of essential oil from dried flowers of *Syzygium aromaticum* showing eugenol (I) , **β-**caryophyllene (II) and eugenyl acetate (III) as major compounds.

Table 2. Chemical constituents of the essential oil of the dried flowers of *Syzygium aromaticum* (clove).

Structure	Relative abundance (%)	Molecular Ion (M^+)	Chemical constituents	Molecular Formula
I	76.97	164	eugenol	$C_{10}H_{12}O_2$
II	9.22	204	**β-caryophyllene**	$C_{15}H_{24}$
III	11.46	206	eugenyl acetate	$C_{12}H_{14}O_3$

being the major constituent, with several biological activities already reported (Asha et al. 2001; Pessoa et al. 2002; Gayoso et al. 2005; Ueda-Nakamura et al. 2006; Braga et al. 2007; Santoro et al. 2007; Shokeen et al. 2008).

Eugenol, 1,8-cineole, and β-selinene (Figure 2 and Table 3) are the major compounds of EOOG and are commonly found in plant species of the Myrtaceae family. Due to their strong insecticidal and ovicidal activity, they are used in many industries, such as flavorings, medicines, pharmaceutical fragrances, cosmetics and pesticide industries (Vivekanandhan et al. 2019).

Harikarnpakdee et al. (2018) identified 16 compounds that represented 100% of the content, the main constituents of this oil were eugenol (67.38%) and *Z*-im-ocimene (14.95%). Such compounds were reported in similar studies performed by: Bunrathep et al. (2007), Matasyohetal)2007), and Malebo et al. (2013). Works published by Ntezurubanza et al. (1987), Zollo et al. (1998), and Zoghbi et al. (2007) presents thymol and γ-terpinene as major components in EOOG.

Turbidimetric Solubility

Turbidity is the cloudiness of a fluid caused by particles in suspension, this property is considered a significant consequence of solid particles in suspension, being considered a quick analysis in determining the solubility using small amounts of the compounds (Kerns and Di 2005; Mazza et al. 2009).

Table 4 shows the turbidity results in NTU, the low turbidity of the lower solution of soluble molecules and a greater turbidity implies a greater distribution of soluble solids, so in the EOSA and EOOG samples the molecules are more available to interact with ethanol and acetone.

The analyzes were performed with DMSO, acetone, ethanol and Tween, because according to Kramer et al. (1983), such organic solvents showed lower toxicity when the larvae of the *Ae. aegypti* mosquito are exposed for 4 h, the LC_{50} values of the solvents were 10, 4.5, 3.8, and 8%, respectively.

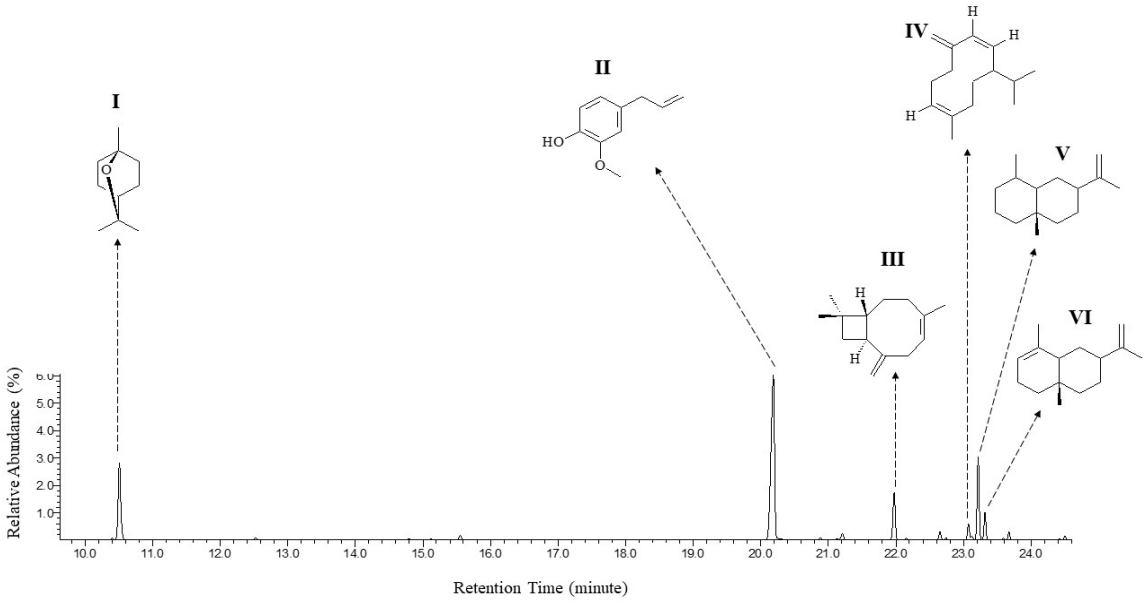

Figure 2. Chromatographic profile of essential oil from dried flowers of *Ocimum gratissimum* showing 1,8-cineole (I), eugenol (II) and β-selinene (V) as major compounds.

Table 3. Chemical constituents present in essential oils *Ocimum gratissimum*.

Structure	Relative abundance (%)	Molecular Ion (M^+)	Chemical constituents	Molecular Formula
I	18.04	154	1,8-cineole	$C_{10}H_{18}O$
II	50.04	164	eugenol	$C_{10}H_{12}O_2$
III	9.50	204	*trans*-caryophyllene	$C_{15}H_{24}$
IV	2.67	236	germacrene D	$C_{15}H_{24}$
V	14.99	204	β-selinene	$C_{15}H_{24}$
VI	4.76	204	α-selinene	$C_{15}H_{24}$

Table 4. Result of turbidimetric analysis of the essential oils.

Oil	Solvent	Turbidity (100 mg/ml)	Turbidity (120 mg/ml)
EOSA	DMSO 1%	7.25 NTU	9.50 NTU
	Acetone 1%	13.75 NTU	14.75 NTU
	Ethanol 1%	30.25 NTU	13.75 NTU
	Tween 1%	2.50 NTU	0.00 NTU
EOOG	DMSO 1%	18.75 NTU	30.25 NTU
	Acetone 1%	91.50 NTU	33.00 NTU
	Ethanol 1%	61.76 NTU	102.00 NTU
	Tween 1%	30.50 NTU	32.00 NTU

Larvicidal Bioassays

It's method for the statistical analysis, the EOSA was used as a control group and the EOOG as a test sample. The LC_{50} data, as well as confidence intervals, probability of significance and the chi-square test (X^2) are presented in Table 5. With *p*-value < 0.05 and calculated X^2 values > than critical X^2, it is possible to verify that there is a statistical relationship between larval mortality and the concentration of EOs, that is, the variables (concentration versus mortality) are totally dependent (Moore et al. 2013).

According to Fayemiwo et al. (2014), EOSA LC_{50} values ranged from 40 to 90 mg/ml. Araujo et al. (2016) and Pandiyan et al. (2019) reported that eugenol is found in the EO in greater relative abundance (on average 65%) and acts significantly on larval mortality. Some variations are observed, such as the date of collection and characteristics of the plant material (dry or wet). It is observed that this factor, together with the use of different populations and types of methodologies, can interfere in the variation of the LC_{50}.

Cheng et al. (2003) showed that LC_{50} values above 100 mg/ml are considered inactive, values between 50 and 100 mg/ml are considered active and those with LC_{50} lower than 50 mg/ml are considered highly active. With this, we can infer that the EOOG qualifies as a potential larvicidal agent. The present observation may be linked to the high relative abundance of 1,8–cineole present in the control sample, this compound being identified for the first time in high concentration in this species.

Conferring the information, the terpenic components, alcohols and aldehydes of the EO are the main responsible for the insecticidal or larvicidal activity (Lee 2006; Lucia et al. 2007). Such compounds may have an important relationship between their chemical structure and biological activity, thus being able to act on digestive and neurological enzymes as well as interacting with the integument of insects (Isman 2000).

At the time, no other work was carried out with the EO of this species. Cavalcanti et al. (2004) and Okigbo et al. (2010) presented results of larvicidal test of extracts against *Ae. aegypti* and *Culex pipens*, the LC_{50} values ranging from 11.4 to 50 mg/ml and contributed with an effect of 70% of mortality.

CONCLUSION

Considering the results obtained, in the present work it can be concluded that the EOSA and EOOG showed larvicidal activity against *Ae. aegypti*. LC_{50} data of 54.6 mg/ml for OG and IC_{50} equal to 6.176 mg/ml corroborate the good activity of 1,8–cineole and eugenol, identified for the first time

Table 5. Lethal concentrations for 50% (LC_{50}) of *Aedes aegypti* larvae against EOSA and EOOG.

EOs	LC_{50} (IC)[a] mg/ml	LC_{90} (IC)[a] mg/ml	Value-p	X^2 calculated
EOSA	41.8 (32.0 – 51.7)	90.2 (88.4 – 101.3)	0.00	27.63
EOOG	62.8 (50.2 – 74.2)	105.2 (94.3 – 110.2)	0.00	45.76

a – Confidence Interval; X^2 – chi square

as a major constituent of *O. gratissimum* species. For a better evaluation of the activity, the solubility conditions of the oil against some solvents were optimized, with ethanol being considered less toxic and with better oil solubility at the concentrations worked, turbidity values around 30 NTU contributed to the evaluation.

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AUTHORS' CONTRIBUTIONS

Material preparation, data collection and analysis were performed by ALS Santos, GTO Silva, AS Nascimento, LFF Lima and FPS Santos. The first draft of the manuscript was written by ALS Santos, CM Feitosa and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

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