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Larvicidal activity of *Ottonia anisum* metabolites against *Aedes aegypti*: A potential natural alternative source for mosquito vector control in Brazil

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ABSTRACT

Background & objectives: Aedes aegypti mosquito is the principal vector of the viruses responsible for urban yellow fever, dengue, dengue haemorrhagic fever, as well as Zika and chikungunya in Brazil. The present study was aimed to investigate the insecticidal potential of the extract and fractions of *Ottonia anisum*, along with special metabolites isolated from it, as natural alternatives against larvae (L3) of *Ae. aegypti*, vector of potentially deadly tropical infections in Brazil.

Methods: The plant species *O. anisum* was collected in March 2015, at Xerém area, in Rio de Janeiro City, Brazil. Crude extracts and the isolated pure compounds were screened for toxicity against *Ae. aegypti* larvae (L3). Bioassays were performed on 20 larvae (L3) of *Ae. aegypti* in triplicate. The samples were dissolved in a mixture of acetone and DMSO at final concentrations of 1–200 µg/ml. The toxicity of the solutions was evaluated towards the growth and development of *Ae. aegypti* larvae till emergence of adults.

Results: The crude hexane extract showed 100% larval mortality 24 h after treatment at a concentration of 200 µg/ml. The bioassays using 1-butyl-3,4-methylenedioxybenzene revealed 100% mortality among L3 larvae, 24 h after the treatment at a concentration of 30 µg/ml, the LC_{50} recorded was 1.6 µg/ml. At concentration of 10 µg/ml, the L3 larval mortality recorded was 92%.

Interpretation & conclusion: The metabolite 1-butyl-3,4-methylenedioxybenzene showed potent toxicity against *Ae. aegypti* larvae. This arylbutanoid agent could be used as a natural alternative adjuvant pesticide, in new compositions that would be environmentally safer.

Key words 1-butyl-3,4-methylenedioxybenzene; *Aedes aegypti*; dengue; larvicidal activity; *Ottonia anisum; Piper*

INTRODUCTION

Dengue is currently the most common mosquito-borne viral infection affecting humans. More than 2.5 billion people are at risk of infection and an estimated 390 million dengue infections occur annually in around 125 countries in tropical and subtropical regions worldwide[1]. Major factors facilitating such expansion include climatic changes, increase in urbanization and international travels. Unfortunately, the non-availability of an efficacious antiviral drug or vaccine and lack of effective vector control strategies make dengue a serious public health concern in tropical and subtropical regions[2]. The mosquito vectors *Aedes aegypti* and *Ae. albopictus* are responsible for the majority of dengue transmissions. Dengue virus is widely distributed in tropical and subtropical zones of the world and several outbreaks have been reported in past years in Asian and Latin American countries. The Southern Cone countries (Argentina, Brazil, Chile, Paraguay and Uruguay) are contributing for majority of the dengue cases in Latin America, with Brazil accounting for almost 98.5% of the fatalities[3].

The Zika virus disease is also transmitted by *Ae. aegypti* and it has spread rapidly within the Americas after an outbreak in Brazil in 2014. In 2015, an increasing number of infants with small head circumference, "microcephaly", was observed in Brazil's Northeast region[4]. In the year 2016, the estimated cases of Zika ranged between 4,40,000 and 13,00,000 in the country. Brazil accounts for 94% of confirmed cases of Zika in the Americas, according to the Pan American Health Organization (2016)[5]. With regard to chikungunya virus, the first case

in Brazil was identified in 2014. Initially restricted to a few municipalities in Northeast region, the virus spread across the country. About 2,63,598 suspected cases were reported nationwide in 2015[5]. The effects of the virus are considered worrisome, causing chronic joints pain that can last for months.

In January 2017, the Brazil Ministry of Health reported 12 suspected cases of yellow fever, another viral disease transmitted by *Ae. aegypti*, from six municipalities in rural areas in the State of Minas Gerais. Later, after an update, a total of 110 suspected cases, including 30 deaths, had been reported from 15 municipalities of Minas Gerais. The serological tests for 19 suspected cases were positive for yellow fever which included 10 deaths[6].

The emergence of a great number of fatal mosquito-borne viral infections and insecticide-resistant mosquitoes has increased the interest in exploring new effective products against adult *Ae. aegypti* mosquitoes as well as its larvae. The synthetic chemical products continue to lose their efficacy against the mosquitoes, compelling the use of higher dosage or different kinds of pesticides to control the mosquito vectors[3]. Because no vaccine has been effective in preventing dengue, the best control measure is to control the adult mosquitoes and eliminate their larval population. Many strategies have been used to control *Ae. aegypti* mosquitoes and their immatures, as well as for the improvement of basic sanitation[7]. However, the indiscriminate use of synthetic insecticides has lead to the emergence of resistant strains of mosquitoes and resulting in an uncontrolled increase in the mosquito population.

In this context, many plant products have been evaluated for their toxic properties against different pests, especially in the form of essential oils (EOs). The EOs possess acute contact and fumigant toxicity to insects, repellent activity, antifeedant activity, as well as development and growth inhibitory activity[8]. The alternative use of EOs has two advantages over other natural pesticides. The first benefit is its low toxicity on mammals, birds and fish. The second advantage is its great structural diversity as a pathway to a potential source of bioactive molecules[9].

Ottonia anisum Sprengel is a shrub commonly found in Southeastern Brazil. This species is sold in open-air markets in the State of Rio de Janeiro and is used in traditional medicine and religious rituals in Northern Brazil due to its anesthetic properties for relieving oral pain and toothache[10-11]. Studies on the chemistry and biological activity of *O. anisum* are sparse and rare. So far, no studies on its insecticidal activity have been conducted. Literature survey showed the isolation and identification of 1-butyl-3,4-methylenedioxybenzene as the major metabolite found in the leaves and the roots from the plant species[12-13]. The six aristolactams including, aristolactam BII, piperolactam C, goniothalactam, stigmalactam, aristolactam AII and aristolactam BIII have been also isolated from roots of this species[14]. Recently, Lopez *et al*[15] reported the isolation and characterization of three amides (pipercallosidine, piperine and valeramide), as well as a mixture of seven amides (valeramide, 4,5-dihydropiperlonguminine, N-isobutil-6-piperonil-2-hexenamide, piperovatine, dihydropipercallosidine, pipercallosidine and pipercallpsine) with anesthetic properties from the roots of *O. anisum*.

The present study was aimed to investigate the insecticidal potential of the crude root extract of *O. anisum*, along with special metabolites isolated from it, as natural alternatives against larvae (L3) of *Ae. aegypti*, the vector of dengue, Zika, urban yellow fever and chikungunya viruses that are potentially deadly tropical infections in Brazil.

MATERIAL & METHODS

Plant material

The plant material was collected in Xerém, Duque de Caxias, state of Rio de Janeiro (RJ), Brazil, in March 2015. The botanical voucher was identified by Dr Elsie Franklin Guimarães from Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, as *O. anisum* Sprengel, and a sample was deposited at the herbarium of the Rio de Janeiro Botanical Garden (JBRJ), with registration under number RB 393494.

General procedure

Silica gel (Merck, 60-200 mesh) was used for column chromatography separations, while analytical TLC was performed using silica gel in 60 PF254 layers (Merck). The solvents for column chromatography separation and for GC-MS and NMR analysis were from Tedia (Brazil).

Phytochemical investigation

Extract preparation and isolation of pure compound 1-butyl-3,4-methylenedioxybenzene: Dried and powdered leaves (300 g) of *O. anisum* were extracted by means of static maceration with *n*-hexane, followed by adding methanol at room temperature. The resulting solutions were filtered separately and the solvent was evaporated under reduced pressure, thereby yielding 11.7 g of *n*-hexane extract and 43.5 g of methanol extract. About 20 g of methanol extract was suspended in a mixture of water and methanol (7:3) and was successively partitioned with

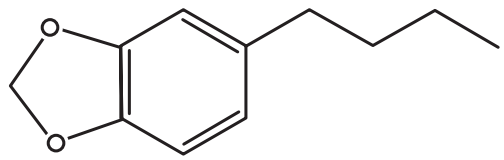

Fig 1. Chemical structure of the major isolated compound 1-butyl-3,4-methylenedioxybenzene isolated from the stem of O. *anisum* Sprengel.

n-hexane, dichloromethane, ethyl acetate and *n*-butanol. The *n*-hexane extract of *O. anisum* was subjected to chromatographic column over silica gel, with a gradient of *n*-hexane, ethyl acetate and methanol as the mobile phase, at increasing polarities. In total, 120 fractions were obtained. Fractions 5–9 were analyzed using thin-layer chromatographic plates and GC-MS, thus enabling characterization of the pure compound 1-butyl-3,4-methylenedioxybenzene. An amount of 1.36 g of the pure compound was obtained through this process. The structure of the pure compound was established through its MS fragmentation pattern (GC-MS analysis), and through ^{1}H and ^{13}C RMN analysis, and the data were compared with the records in the literature[13]. The structure of the compound identified is shown in Fig. 1.

GC-MS analysis

Qualitative analyses were carried out in a Shimadzu GC-QP2010 PLUS machine equipped with a ZB-5MS fused silica capillary column (30 m × 0.25 mm × 0.25 mm film thickness). The operating temperatures used were: injector 270°C, detector 290°C and column oven from 60 to 290°C (10°C min^{-1}). Helium at 1 ml min^{-1} was used as a carrier gas. The pure compound was identified through comparison of its mass spectra and fragmentation pattern with published data[13] and through computer matching with the WILEY 275 and the National Institute of Standards and Technology (NIST 3.0) libraries that were provided with the computer, controlling the GC-MS system.

Nuclear magnetic resonance spectroscopy

The pure compound obtained from stems of *O. anisum* was analyzed using ^{1}H and ^{13}C-NMR and the data were recorded on a Varian VNMRS 500 spectrometer. The chemical shifts were determined through DMSO-d_6, using tetramethylsilane (TMS) as the internal standard. The signals of the NMR analyses were compared with the data available in literature[13].

Bioassays

Aedes aegypti eggs were obtained from the Sentinel Operational Center for Vector Mosquitoes of the Oswaldo Cruz Institute, Fiocruz, RJ, and the bioassays were done in the Insect Vector Laboratory, Severino Sombra University, Vassouras, RJ. The bioassays were carried out using eggs that were placed in a receptacle containing mineral water with fish food (0.3 mg/larva) (Alcon Guppy® Alcon, Camboriú, SC, Brazil) for hatching[16–17]. All the experiments to determine the effects of 1-butyl-3,4-methylenedioxybenzene from *O. anisum* and its methanol and hexane extracts were carried out on III instar (L3) larvae, according to methodology by WHO[18]. The isolated compound 1-butyl-3,4-methylenedioxybenzene and the extracts were dissolved in DMSO and acetone (1:1) at a final concentrations of 1–200 µg/ml. In the larval treatment groups, with 20 larvae per group, extracts were added to glass containers (4 × 4.5 cm) containing mineral water (20 ml) at final concentrations of 10, 100 and 200 µg/ml. The larval treatments consisted of adding the substance to glass containers (2 × 4 cm) containing mineral water (20 ml) at final concentrations of 1, 10, and 30 µg/ml. The *Ae. aegypti* larval groups (L3) were evaluated in triplicate with three repetitions, as described elsewhere[16-20] and adopted from WHO[21]. Two control groups were included: One with a DMSO and acetone solution (without extracts or substance) (Testimony) and another with untreated solution. The bioassays were performed in a climate-controlled chamber at 28 ± 1 °C temperature and 70 ± 10% relative humidity, with 12 h photoperiods throughout the experiments, and the toxicity against *Ae. aegypti* larvae and their growth and development was evaluated till adult emergence[18–21].

Statistics

The data were analyzed using the ANOVA F-test[22] and the χ^2-test, in which $p \leq 0.05$ and $p \leq 0.01$ were considered significant, respectively. Standard deviations were calculated using the averages from the experiments using GraphPad Instat 3.05[23] and trimmed Spearman-Karber analysis, in order to determine the LC_{50}[24].

RESULTS

The phytochemical investigation confirmed that *O. anisum,* isolated as the major constituent from the methanol extract (>1 g from *n*-hexane leaves fraction) is a valuable source of the arylbutanoid compound 1-butyl-3,4-methylenedioxybenzene, present in almost all the organs of this plant species. The bioassays performed using the crude methanol extract showed that this interfered with the duration of development in the pupal stage (9.3 ± 2.1 days; $p < 0.001$) and the L3-adult stage (12.3 ± 3.2 days; $p < 0.001$), at the concentration of 200 µg/ml (Table 1a).

Table 1. Duration of development (a), viability (b) and mortality (c) among *Aedes aegypti* larvae (L3) treated through culturing with a methanol extract (mg/ml) *of Ottonia anisum*

(a)	Larvae (Days)		Pupae (Days)		Emergence (Days)	
Treatment	$\overline{X}$ ± SD	VI	$\overline{X}$ ± SD	VI	$\overline{X}$ ± SD	VI
Control	3 ± 0.7a	2–5	12.5 ± 3.6a	8–19	16.2 ± 3.7a	9–20
Testimony	3.2 ± 0.6a	2–6	12.1 ± 3a	7–17	15.7 ± 3.3a	9–19
10	3.1 ± 1.7a	2–13	10.1 ± 2.3c	7–16	15.1 ± 3.3b	9–18
100	3.4 ± 1.9a	2–8	10.3 ± 2.2c	7–13	12.7 ± 3c**	8–16
200	3.1 ± 1a	2–8	9.3 ± 2.1d***	6–14	12.3 ± 3.2c***	8–17

(b)	L3–L4		L4–Pupae		Pupae		L3–Adult	
	$\overline{X}$ ± SD	%	$\overline{X}$ ± SD	%	$\overline{X}$ ± SD	%	$\overline{X}$ ± SD	%
Control	20 ± 0a	100	20 ± 0a	100	20 ± 0	98	19.7 ± 0.6a	98
Testimony	20 ± 0a	98	19.7 ± 0.7a	98	19 ± 1	91	17.6 ± 2.5a	88
10	20 ± 0a	92	18.7 ± 2.3a	55	9.7 ± 4.1b	100	9.7 ± 4.1b	47
100	20 ± 0a	87	17.3 ± 2.1a	52	9 ± 1bc*	82	7.3 ± 3.8b*	37
200	20 ± 0a	97	19.3 ± 0.6a	78	15 ± 4.5ac*	98	14.7 ± 5.1a	73

(c)	L3			L4			Pupae		
	$\overline{X}$ ± SD	VI	%	$\overline{X}$ ± SD	VI	%	$\overline{X}$ ± SD	VI	%
Control	0	0	0	0	0	0	0.3 ± 0.7a	17–17	2
Testimony	0.7 ± 1.1a	3–3	2	0.3 ± 0.7a	16–16	2	1.3 ± 2.3a	17–18	9
10	1.7 ± 2.8a	3–5	8	8.3 ± 6.1a	7–16	45	0	0	0
100	2.6 ± 2.1a	3–5	13	8.3 ± 1.5a	14–15	48	1.7 ± 0.7a	14–14	18
200	0.3 ± 0.7a	3–5	3	4.3 ± 4.1a	5–16	22	0.3 ± 0.7a	14–14	2

Experiments with 20 larvae (L3) of *Ae. aegypti*, for each test group and control, in triplicate (n = 60); Mean and standard deviation ($\overline{X}$ ± SD); Range of variation (VI); Values followed by the same letter (a = a, b = b, c = c) did not present any significant difference; Significance level according to the Tukey's test is represented as ***$p < 0.001$, **$p \leq 0.01$, *$p < 0.1$ *vs* DMSO and acetone control (1:1) (Testimony).

Regarding viability, the results from the same extract at a concentration of 100 µg/ml showed that 52% of the fourth-stage (L4) larvae and 82% of the pupae were viable, and that 37% of the L3 larvae reached adult stage (Table 1b). From the crude methanol extract at the concentrations of 10 and 100 µg/ml, respectively, the larval mortality rates (L3 + L4) were 53 and 61%, respectively (Table 1c). At the concentration of 100 µg/ml, pupal mortality recorded was 18% (Table 1c).

The hexane extract of *O. anisum* interfered with larval development (4.1 ± 2.8 days; $p < 0.1$) and L3-adult development/emergence (9 ± 0 days; $p < 0.1$) of *Ae. aegypti,* at the concentration of 100 µg/ml (Table 2a). At the same concentration, the larval viability was 12% (2 ± 3.5 days; $p < 0.001$), and only 3% of these emerged as adult (Table 2b). The same extract at a concentration of 100 µg/ml caused third-stage larval mortality of 72% after 48 h and fourth-stage larval mortality of 25%. This extract at a concentration of 200 µg/ml, caused 100% larval mortality 24 h after the treatment (Table 2c).

The bioassays performed using 1-butyl-3,4-methylenedioxybenzene at the concerntration of 10 µg/ml showed that this interfered with the duration of development in the larvae (2 ± 0 days; $p<0.001$); pupae (7.2±0.5 days; $p<0.001$); and the L3-Adult stages (9±0 days; $p<0.001$) (Table 3a). The larvae of *Ae. aegypti* treated with 1-butyl-3,4-methylenedioxybenzene at a concentration of 10 µg/ml showed 8% viability at the larval stage (L3) and 60% at the pupal stage, and 5% of the L3 larvae reached adult stage (L3-adult) (Table 3b). Treatment with the isolate 1-butyl-3,4-methylenedioxybenzene at a concentration of 1 µg/ml resulted in 44% larval mortality(L3 + L4) (Table 3c). At a concentration of 10 µg/ml, the third-stage larval and pupal mortality was 92 and 40%, respectively (Table 3c). At the highest concentration tested (30 µg/ml), the L3 larval mortality was 100%, 24 h after the treatment. This treatment showed LC_{50} = 1.6 µg/ml (lower 95% confidence limit = 0.7 µg/ml and upper limit = 3.3 µg/ml) (Table 3c).

DISCUSSION

Several species of the genus *Piper* have been investigated regarding their insecticidal activity[3]. In India, species like *Piper longum*, *P. betle* and *P. cubeba* have shown insecticidal activity against mosquitoes and flies[25]. Phytochemical studies on *Piper* species have shown the presence of a variety of bioactive metabolites, including alkaloids, chromenes, amides, flavonoids and terpenoids[26]. These compounds have several modes of action, including contact toxicity, synergism, repellent and antifeedant properties[27].

Table 2. Duration of development (a), viability (b) and mortality (c) among *Ae. aegypti* larvae (L3) treated through culturing with *n*-hexane extract (mg/ml) of *O. anisum*

(a)	Larvae (Days)		Pupae (Days)		Emergence (Days)	
Treatment	$\overline{X}$ ± SD	VI	$\overline{X}$ ± SD	VI	$\overline{X}$ ± SD	VI
Control	2.9 ± 0.7a	2–5	12.5 ± 3.5a	8–19	16.2 ± 3.7a	9–20
Testimony	3 ± 0.6a	2–6	12 ± 3a	7–17	15.7 ± 3.3a	9–19
10	2.7 ± 1a	2–6	16.1 ± 3.7b***	8–20	18.2 ± 3.8a	9–21
100	4.1 ± 2.8b*	2–9	8 ± 0a	8–8	9 ± 0b*	9–9
200	0	0	0	0	0	0

(b)	L3–L4		L4–Pupae		Pupae		L3–Adult	
	$\overline{X}$ ± SD	%	$\overline{X}$ ± SD	%	$\overline{X}$ ± SD	%	$\overline{X}$ ± SD	%
Control	20 ± 0a	100	20 ± 0a	100	20 ± 0a	98	19.7 ± 0.6a	98
Testimony	20 ± 0a	98	19.7 ± 0.7a	98	19 ± 1a	91	17.6 ± 2.5a	88
10	20 ± 0a	92	19.7 ± 0.6a	11	5.3 ± 4.5b***	17	2.7 ± 2.1b***	2
100	20 ± 0a	28	2 ± 3.5b***	12	0.7 ± 1.1c***	100	0.7 ± 1.1b***	3
200	20 ± 0a	0	0	0	0	0	0	0

(c)	L3			L4			Pupae		
	$\overline{X}$ ± SD	VI	%	$\overline{X}$ ± SD	VI	%	$\overline{X}$ ± SD	VI	%
Control	0	0	0	0	0	0	0.3 ± 0.7a	17–17	2
Testimony	0.7 ± 1.1a	3–3	2	0.3 ± 0.7a	16–16	2	1.3 ± 2.3a	17–18	9
10	0.3 ± 0.7a	3–5	8	14.7 ± 3.5b***	7–14	8	0	0	0
100	18 ± 3.5b***	2–3	72	1.3 ± 2.3a	2–14	25	0	0	0
200	20 ± 0b***	1–1	100	0	0	0	0	0	0

Experiments with 20 larvae (L3) of *Ae. aegypti*, for each test group and control, in triplicate (n = 60); Mean and standard deviation ($\overline{X}$ ± SD); Range of variation (VI); Values followed by the same letter (a = a, b = b, c = c) did not present any significant difference; Significance level according to the Tukey's test is represented as ***p <0.001, **p ≤0.01, * p<0.1 *vs* DMSO and acetone control (1:1) (Testimony).

Table 3. Duration of development (a), viability (b) and mortality (c) among *Ae. aegypti* larvae (L3) treated through culturing with 1-butyl-3,4-methylenedioxybenzene (mg/ml), isolated from the hexane fraction of stems of *O. anisum*

(a)	Larvae (days)		Pupae (days)		Emergence (days)	
Treatment	$\overline{X}$ ± SD	VI	$\overline{X}$ ± SD	VI	$\overline{X}$ ± SD	VI
Control	6.2 ± 2.8a	4–11	15.3 ± 3.3a	8–19	17.3 ± 2.7a	10–23
Testimony	8.6 ± 1.9b	4–12	17.1 ± 2.4b	7–17	20 ± 2.8b	9–24
1	8.1 ± 2.6b	4–11	11.4 ± 2.9c***	8–20	15.4 ± 1.6c***	9–16
10	2 ± 0c***	2–2	7.2 ± 0.5c***	7–8	9 ± 0d***	9–9
30	0	0	0	0	0	0

(b)	L3–L4		L4–Pupae		Pupae		L3–Adult	
	$\overline{X}$ ± SD	%	$\overline{X}$ ± SD	%	$\overline{X}$ ± SD	%	$\overline{X}$ ± SD	%
Control	20 ± 0a	95	19.3 ± 1.1a	98	19 ± 0a	100	18.6 ± 1.5a	93
Testimony	20 ± 0a	97	19.3 ± 1.1a	100	19.3 ± 1.1a	100	19.3 ± 1.1a	97
1	20 ± 0a	83	16.7 ± 3.5a	38	6.3 ± 1.5b***	100	6.3 ± 1.5b***	32
10	20 ± 0a	8	1.7 ± 1.5b***	100	1.7 ± 1.5c***	60	1 ± 1c***	5
30	20 ± 0a	0	0	0	0	0	0	0

(c)	L3			L4			Pupae		
	$\overline{X}$ ± SD	VI	%	$\overline{X}$ ± SD	VI	%	$\overline{X}$ ± SD	VI	%
Control	1 ± 1a	7–7	5	0.3 ± 0.6	8–8	2	0	0	0
Testimony	0.6 ± 1.1a	7–7	3	1 ± 1	16–16	0	0	0	0
1	3.3 ± 3.5a	11–11	17	5.3 ± 5.5	7–14	27	0	0	0
10	18.3 ± 1.5b***	1–1	92	0	0	0	0.7 ± 0.6b*	8–9	40
30	20 ± 0c***	1–1	100	0	0	0	0	0	0

Experiments with 20 larvae (L3) of *Ae. aegypti*, for each test group and control, in triplicate (n = 60); Mean and standard deviation ($\overline{X}$ ± SD); Range of variation (VI); Values followed by the same letter (a = a, b = b, c = c) did not present any significant difference; Significance level according to the Tukey's test is represented as ***p <0.001, **p ≤0.01, *p <0.1 *vs* DMSO and acetone control (1:1) (Testimony).

Marques and Kaplan[3] reported that most of the studies on *Piper* and *Ae. aegypti* refer to the active extract, essential oil and/or pure isolated secondary metabolites of *P. nigrum*. With reference to essential oils, the literature on the genus *Piper* shows a great variety of species with active essential oils. *Piper aduncum* is the species with essential oil showing significant larvicidal properties against *Ae. aegypti,* that has been extensively studied. The arylpropanoid dillapiole-rich chemotype species has been shown to be very efficient against L3 larvae of *Ae. aegypti*[28].

The previous studies by Marques *et al*[13-14], have reported about the isolation and characterization of methylenedioxybenzene derivatives (1-butyl-3,4-methylenedioxybenzene) from the leaves and roots of *O. anisum* Sprengel. This arylbutanoid compound was characterized as the major compound present in this species, especially in the leaves and stems, with yield of > 1 g (w/w). Moreira *et al*[12] also reported that the 1-butyl-3,4-methylenedioxybenzene was the main constituent of the essential oil from *O. anisum* leaves, accounting for up to 95% of the volatile components.

The larvicidal activity of 1-butyl-3,4-methylenedioxybenzene isolated from *O. anisum* is in agreement with Nascimento *et al*[29] who reported results from chemical and insecticidal investigations on the essential oils from *P. klotzschianum*. The major chemical constituent identified from this plant species was again the metabolite 1-butyl-3,4-methylenedioxybenzene, which accounted for 96.19% of the essential oil from the roots; 84.75% from the stems; 81.04% from the leaves and 36.92% from the seeds. The LC_{50} of the crude essential oil against IV-instar *Ae. aegypti* larvae was observed for the seeds (LC_{50} = 13.27 µg/ml) and for the roots (LC_{50} = 10 µg/ml).

The evident pronounced biosynthesis of this substance can be related to the defense mechanisms of the plant against pathogens and to a large variety of aggression from natural external agents[30]. Phenylpropanoids and their derivatives are known to contribute to plant mechanism responses towards biotic and abiotic factors such as ozone exposure, low nutrient levels, pathogen attacks (fungal, bacterial, and viral) and herbivore attack[31]. Many of these compounds have been shown to be active against the vector of dengue virus. Dias and Moraes[32] described many simple pure phenylpropanoids that were active against *Ae. aegypti*. The C6–C1 benzyl salicylate was found to be an active phenolic compound with a low LC_{50} of 6.8 µg/ml, followed by the C6–C1 compound (*E*)-cinnamaldehyde (LC_{50} = 24.4 µg/ml) and the isomers (*E*)-asarone with LC_{50} = 27 µg/ml and (*Z*)-asarone, which were more active, with LC_{50} = 16 µg/ml. Simple methylenedioxy phenylpropanoids such as safrole, the chemical marker of *P. hispidinervum,* were active with LC_{50} = 9.88 µg/ml. Similar structural methylenedioxy phenylpropanoids such as eugenol and methyl eugenol were also active, with LC_{50} of 44.5 µg/ml and 57.65 µg/ml, respectively. The presence of a methylenedioxy moiety in phenylpropanoids contributes towards increasing the activity of safrole, in comparison with similar structural compounds such as eugenol and methyl eugenol. The methylenedioxy phenyl (MDP) moiety is a structural feature found in many natural occurring special metabolites, especially in the alkaloids, amides and phenylpropanoids found in *Piper* species. This MDP moiety is present in 1-butyl-3,4-methylenedioxybenzene and also in all aristolactams isolated from the roots of *O. anisum*. The insects are the preferential target species for these plant allelochemicals. Murray[31] described a possible mechanism of action for these metabolites in insects consisting of modulation of the insect cytochrome P-450 (CYP) pathways to enhance or synergize the toxicity of the primary allelochemical deterrent, thereby avoiding concomitant plant damage. Many studies have shown that the compounds such as *Piper* amides and methylenedioxybenzene derivatives undergo oxidative biotransformation through CYP insect enzymes, thus leading to formation of an intermediate inhibitory metabolite complex. The toxic effect of these metabolites is mediated through modulation of the processes of inhibition and induction of the insect CYP[31]. The phenylpropanoid methysticin (isolated from *P. methysticum*), the amide piperine (isolated from *P. nigrum*) and the more complex MDP-substituted agents podophyllotoxin and tetrahydroberberine have been shown to enhance the toxicity of pyrethroids and carbamates in several insect species[25, 33]. Many studies have described the toxic effect of lignans, aristolochic acid and amides containing the MDP moiety[34-36]. Recent studies by Cabral *et al*[16] and Leite *et al*[19] on the neolignan grandisin (isolated from *P. solmsiamum*) showed similar toxicity against *Ae. aegypti* at concentrations of 150 and 200 µg/ml. Maleck *et al*[20] confirmed that piperamides isolated from *P. tuberculatum* and *P. scutifolium* presented larvicidal activity, with LD_{50} of 155.5 and 50 µg/ml, respectively. In the present study the phenylpropanoid derivative *i.e.* 1-butyl-3,4-methylenedioxybenzene was shown to be a potential natural alternative pesticide since it is a volatile compound that is less persistent on surfaces and is degraded more rapidly. The major active compound, 1-butyl-3,4-methylenedioxybenzene, is responsible for more than 10% of *n*-hexane extract and easily isolated in high purity level. Due to these facts, the economical use

of this natural source could be viable and the sustainable cultivation of this native Brazilian species by the communities may be encouraged.

CONCLUSION

The study findings suggest that the metabolite, 1-butyl-3,4-methylenedioxybenzene could be used as a natural alternative adjuvant pesticide, in new compositions that would be environmentally safer than some synthetic insecticides. The bioassays using this metabolite and enriched fractions revealed significant toxicity among L3 larvae after 24 h after the treatment at low concentrations.

In addition, the extraction of this natural active compound from Brazilian native shrub species may be a sustainable economic alternative against the tropical viruses such as: dengue, zika, urban yellow fever and chikungunya vector, and may contribute towards reducing the extensive use of toxic pesticides in developing countries with high rates of these viral infections.

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Conflict of interest

The authors report no conflict of interest.

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