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ORIGINAL ARTICLE

LARVICIDAL ACTIVITY OF *Caesalpinia ferrea* MART. AND *Lippia origanoides* CHAM. AGAINST *Aedes aegypti* (LINNAEUS, 1762) (DIPTERA: CULICIDAE)

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ABSTRACT

The control of *Aedes aegypti* has been considered one of the most important public health challenges worldwide. Chemical compounds have long been used for this purpose, but resistance to these molecules has also increased. Therefore, over the last few years several studies have focused on the development of alternative tools, particularly those based on plant metabolites. The purpose of this study was to assess the larvicidal activity of *Caesalpinia ferrea* and *Lippia origanoides* against *Ae. aegypti*. Larvae (L_3) of *Ae. aegypti* Liverpool and Rockefeller strains, as well as of the Recife population were exposed to different concentrations of *C. ferrea* (ranging from 13.1 to 105 mg/mL) and *L. origanoides* (ranging from 16.3 to 130 mg/mL), and the mortality rate was evaluated up to 48 hours after the beginning of the experiment. All tested groups and control group were quadruplicated. For *C. ferrea,* mortality ranged from 42.5% to 100% for *Ae. aegypti* Liverpool strain, from 67% to 100% for *Ae. aegypti* Rockfeller strain, and 57% to 100% for *Ae. aegypti* Recife population after 48 hours of larval exposure. For *L. origanoides*, the larvicidal activity ranged from 75% to 100% for *Ae. aegypti* Liverpool strain, from 61.5% to 100% for *Ae. aegypti* Rockfeller strain, and from 60.5% to 100% for *Ae. aegypti* Recife population. The hydro ethanol extract of *C. ferrea* and *L. origanoides* presented larvicidal activity against *Ae. aegypti*.

KEY WORDS: *Aedes aegypti*; hydro ethanol extracts; *Caesalpinia ferrea*; *Lippia sidoides*; botanical insecticide; mosquitoes.

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INTRODUCTION

Aedes aegypti (Diptera: Culicidae) has been considered one of the most important vectors to cause arthropod-borne diseases, such as Dengue, Zika, Chikungunya and urban Yellow Fever (Braack et al., 2018; Huang et al., 2019) in humans as well as in animals, as in the case of dirofilariasis (Ledesma et al., 2019). In recent years, the number of arbovirus disease cases has increased throughout the world (Marchi et al., 2018). For instance, in Brazil approximately 244,068 suspected cases were registered during the first trimester of 2019 (MS, 2019).

The reduction in arboviral diseases is known to be directly related to vector control, which has, up to the moment, been accomplished using chemical compounds (e.g., organochlorides, organophosphates and pyrethroids) (Manjarres-Suarez et al., 2013). However, the inadequate use of these products has led to vector resistance (Smith et al., 2016; Brito et al., 2016; Valle et al., 2019). The search for alternative mosquito control substances has shown some results, and secondary metabolites from plants with insecticide properties have proved to be promising alternatives (Pino et al., 2013; Gonçalves et al., 2018; Maggi et al., 2018; Pavela et al., 2019). For instance, several botanical compounds, such as terpenes, polyphenols, alkaloids, sterols, saponins and flavonoids have been isolated and their larvicidal potential assessed (Silva et al., 2008; Rawani et al., 2014; Mar et al., 2018; Pavela et al., 2019). In fact, these molecules may inhibit larval development, affecting the intestinal epithelium and anal gills, while some of them present neurotoxic effects (Perumalsamy et al., 2013; Wang et al., 2019). In addition, botanical larvicides have demonstrated low toxicity to non-target organisms as well as to the environment (Masetti, 2016; Wang et al., 2019).

Brazil presents a wide diversity of plants used in phytotherapy, most of which are found in the Northeastern region. For instance, *Caesalpinia ferrea, Lippia gracilis* and *Lippia origanoides* have been widely studied and their antiparasitic, repellent and larvicidal properties analyzed (Silva et al., 2008; Cavalheiro et al., 2009; Vera et al., 2014; Fernandes et al., 2016; Soares et al., 2017). Several phytochemical compounds have been isolated from plants (e.g., *C. ferrea* - flavonoids, saponins, tannins, coumarins, sterols and phenolic compounds; *L. gracilis* and *L. sidoides* - terpenes - carvacrol and thymol) presenting the properties mentioned above (Carvalho et al., 2003; Cavalcanti et al., 2004; Silva et al., 2008; Cavalheiro et al., 2009).

Therefore, the purpose of this study was to assess the larvicidal activity of the hydro ethanol extracts of *C. ferrea* and *L. origanoides* against *Ae. aegypti.*

MATERIAL AND METHODS

Ethical aspects

The Ethical Committee of Animal Experimentation of the Federal Rural University of Pernambuco (license number: 146/2018) approved all procedures herein performed.

Sampling and plant identification

Two species of plants (*Caesalpinia ferrea* Mart. (Fabaceae) and *Lippia origanoides* Cham. (Verbenaceae) were used in this study, both obtained in the State of Pernambuco, Northeastern Brazil. For the identification of *C. ferrea*, branches with leaves and fruit were used. The voucher specimens were deposited in the Geraldo Mariz Herbarium of the Federal University of Pernambuco, under registration number 69656. The identification of *L. origanoides* was performed using branches with leaves and flowers and then deposited in the Dárdano de Andrade Lima Herbarium of the Agronomic Institute of Pernambuco, under registration number 92547.

Hydro ethanol extract preparation

The hydro ethanol extracts were prepared using fruit and leaves, from *C. ferrea* and *L. origanoides* respectively, since these have shown a highly larvicidal potential (Cavalcanti et al., 2004; Cavalheiro et al., 2009). *C. ferrea* fruit and *L. origanoides* leaves were dried using an air circulating drying oven at 40ºC and then ground. Next, both products were kept in 70% ethanol for 72 hours (Matos, 2009). Solutions were filtered and concentrated in a rotatory evaporator under reduced pressure, at 45ºC, for solvent elimination. Both the *C. ferrea* and *L. origanoides* extracts were kept at 4ºC for 24 hours and then lyophilized. Subsequently, the extracts were suspended in distilled water.

Aedes aegypti

Ae. aegypti eggs from the Liverpool and Rockfeller strains (reference strains), and from the Recife population (wild strain) were obtained from colonies kept under controlled temperature (28°C±2ºC), relative air humidity (80%±10%) and a dark-light photoperiod of 12:12h. Shortly after this, eggs were transferred to plastic boxes (2 L volume) containing 1.5 L of dichloride water for egg hatching. Larvae were fed daily with commercial fish food (150 mg). Third instar larvae (L_3) were used for the experiments. All larvae were deprived of food for 24 hours before the assessment.

Larvicidal assessment

A total of 3,600 (L_3) specimens, being 1,200 of each strain/population, were used in this study. 500 larvae were used for the tested groups (100 for each plant extract concentration), while 100 were used for the control groups. Each assessment was performed in quadruplicate (25 L_3 for repetition) for all groups evaluated (different concentrations and controls).

Larvae were exposed to different concentrations of hydro ethanol extracts of *C. ferrea* (13.1 mg/mL, 26.3 mg/mL, 52.5 mg/mL, 78.8 mg/mL and 105 mg/mL) and *L. origanoides* (16.3 mg/mL, 32.5 mg/mL, 65 mg/mL, 97.5 mg/mL and 130 mg/mL). The control groups were exposed to distilled water. All procedures performed followed the methodology recommended by the World Health Organization, with slight modifications (WHO, 2005).

Larvae mortality was assessed at 2h, 4h, 6h, 12h, 24h and 48h after exposure. In addition, dead L_3 were studied under an optical microscope at different magnifications (4 and 10X) to detect physical damage caused by the toxic action of the extracts.

Data analysis

Data went through descriptive statistical analysis to evaluate larvicidal activity. The mortality along the time periods was assessed using the Friedman test, whereas the mortality at different concentrations or among strain/population was evaluated using the Kruskal-Wallis test. Differences between both plant extracts were analyzed using the Mann-Whitney test.

The median lethal concentration (LC_{50}) was calculated by Probit analysis using the mortality percentage at confidence interval of 95%. The significance level was set at 5%. Softwares IBM SPSS version 23 and Medcalc version 14.8.1 were used for the analyses.

RESULTS

The larvicidal activity of *C. ferrea* (Table 1) and *L. origanoides* (Table 2) against each strain/population of *Aedes aegypti* was enhanced with increased concentrations and along the time period studied, but there was no statistical difference ($p > 0.05$). All larvae in the control groups remained alive until the end of the experiment (Tables 1 and 2). Initial larvae mortality was noted 2 hours after exposure for both extracts. For *C. ferrea* at the highest concentration (105 mg/mL) all the reference larvae and wild strain larvae were dead 2 hours after exposure. Meanwhile, for *L. origanoides* at the highest concentration (130 mg/mL) all reference larvae strains were dead 2 hours after exposure, whereas in the wild strain, total mortality was noted 6 hours after exposure (Tables 1 and 2).

Table 1. Larvicidal activity (% ± sd) of *Caesalpinia ferrea* against *Aedes aegypti*.

Ae. aegypti	Concentration (mg/mL)	(% ± sd)						
		2h	4h	6h	12h	24h	48h	p value
Liverpool strain	Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
	13.1	0.0 ± 0.0	0.0 ± 0.0	12.5 ± 3.5	16.5 ± 5.0	27.5 ± 5.0	42.5 ± 2.1	$p^{(1)} = 0.075$
	26.3	3.5 ± 2.1	8.5 ± 2.1	22.0 ± 2.8	31.5 ± 0.7	42.0 ± 0.7	51.0 ± 1.4	$p^{(1)} = 0.075$
	52.5	32.0 ± 9.9	47.5 ± 3.5	56.0 ± 1.4	68.0 ± 1.4	83.5 ± 1.4	99.0 ±1.4	$p^{(1)} = 0.075$
	78.8	95.5 ± 0.7	100 ± 0.0					$p^{(1)} = 0.075$
	105	100 ± 0.0						$p^{(1)} = 1.000$
	p value	$p^{(2)} = 0.065$	$p^{(2)} = 0.064$	$p^{(2)} = 0.066$	$p^{(2)} = 0.066$	$p^{(2)} = 0.066$	$p^{(2)} = 0.081$	
Rockfeller strain	Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
	13.1	9.0 ± 1.4	17.5 ± 3.5	34.0 ± 5.7	49.5 ± 7.8	55.0 ± 7.8	67.0 ± 4.2	$p^{(1)} = 0.075$
	26.3	29.5 ± 0.7	39.0 ± 7.1	50.5 ± 0.7	59.5 ± 6.4	64.5 ± 6.4	73.5 ± 5.0	$p^{(1)} = 0.075$
	52.5	32.5 ± 3.5	56.0 ± 8.5	78.5 ± 5.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	$p^{(1)} = 0.075$
	78.8	95.5 ± 0.7	100 ± 0.0					$p^{(1)} = 0.075$
	105	100 ± 0.0						$p^{(1)} = 1.000$
	p value	$p^{(2)} = 0.071$	$p^{(2)} = 0.066$	$p^{(2)} = 0.066$	$p^{(2)} = 0.069$	$p^{(2)} = 0.069$	$p^{(2)} = 0.069$	
Recife population	Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
	13.1	0.0 ± 0.0	9.5 ± 0.7	23.5 ± 7.8	36.5 ± 3.5	52.5 ± 3.5	57.0 ± 4.2	$p^{(1)} = 0.075$
	26.3	24.5 ± 0.7	34.0 ± 0.0	42.5 ± 3.5	54.0 ± 5.7	60.5 ± 5.7	72.0 ± 2.8	$p^{(1)} = 0.075$
	52.5	31.5 ± 2.1	49.0 ± 1.4	67.5 ± 0.7	79.0 ± 1.4	89.5 ± 1.4	100 ± 0.0	$p^{(1)} = 0.075$
	78.8	88.0 ± 2.8	97.5 ± 3.5	100 ± 0.0				$p^{(1)} = 0.119$
	105	100 ± 0.0						$p^{(1)} = 1.000$
	p value	$p^{(2)} = 0.065$	$p^{(2)} = 0.070$	$p^{(2)} = 0.066$	$p^{(2)} = 0.066$	$p^{(2)} = 0.066$	$p^{(2)} = 0.065$	

(% ± sd): mean mortality percentage ± standard deviation; (1): Friedman Test; (2): Kruskal Wallis Test.

Table 2. Larvicidal activity (% ± sd) of *Lippia origanoides* against *Aedes aegypti*.

Ae. aegypti	Concentration (mg/mL)	(% ± sd) 2h	4h	6h	12h	24h	48h	p value
Liverpool strain	Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
	16.3	0.0 ± 0.0	17.5 ± 3.5	31.0 ± 4.2	39.5 ± 0.7	65.0 ± 0.7	75.0 ± 5.7	$p^{(1)} = 0.075$
	32.5	0.0 ± 0.0	26.0 ± 1.4	42.0 ± 2.8	52.0 ± 1.4	64.0 ± 1.4	81.5 ± 2.1	$p^{(1)} = 0.075$
	65.0	33.0 ± 4.2	42.0 ± 4.2	55.0 ± 1.4	70.5 ± 2.1	93.0 ± 2.1	100 ± 0.0	$p^{(1)} = 0.075$
	97.5	92.5 ± 3.5	100 ± 0.0					$p^{(1)} = 0.075$
	130	100 ± 0.0						$p^{(1)} = 1.000$
	p value	$p^{(2)} = 0.064$	$p^{(2)} = 0.066$	$p^{(2)} = 0.066$	$p^{(2)} = 0.066$	$p^{(2)} = 0.066$	$p^{(2)} = 0.065$	
Rockfeller strain	Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
	16.3	0.0 ± 0.0	20.5± 0.7	41.0 ± 2.8	53.0 ± 5.7	57.5 ± 5.7	61.5 ± 2.1	$p^{(1)} = 0.075$
	32.5	0.0 ± 0.0	32.0 ± 4.2	44.0 ± 5.7	52.5 ± 3.5	56.5 ± 3.5	62.0 ± 2.8	$p^{(1)} = 0.075$
	65.0	40.5 ± 0.7	50.5 ± 2.1	75.5 ± 0.7	92.0 ± 2.8	98.5 ± 2.8	100 ± 0.0	$p^{(1)} = 0.075$
	97.5	93.0 ± 1.4	100 ± 0.0					$p^{(1)} = 0.075$
	130	100 ± 0.0						$p^{(1)} = 1.000$
	p value	$p^{(2)} = 0.064$	$p^{(2)} = 0.066$	$p^{(2)} = 0.076$	$p^{(2)} = 0.079$	$p^{(2)} = 0.079$	$p^{(2)} = 0.078$	
Recife population	Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
	16.3	0.0 ± 0.0	20.5 ± 0.7	34.0 ± 7.1	42.0 ± 2.8	50.5 ± 2.8	60.5 ± 0.7	$p^{(1)} = 0.075$
	32.5	19.5 ± 2.1	30.0 ± 0.0	39.0 ± 0.0	45.0 ± 5.7	67.0 ± 5.7	74.5 ± 3.5	$p^{(1)} = 0.075$
	65.0	42.0 ± 2.8	51.5 ± 2.1	61.5 ± 3.5	71.0 ± 8.5	81.0 ± 8.5	98.5 ± 2.1	$p^{(1)} = 0.075$
	97.5	47.0 ± 1.4	61.5 ± 2.1	68.5 ± 2.1	78.0 ± 2.8	100 ± 0.0		$p^{(1)} = 0.075$
	130	67.5 ± 3.5	82.5 ± 3.5	100 ± 0.0				$p^{(1)} = 0.075$
	p value	$p^{(2)} = 0.067$	$p^{(2)} = 0.067$	$p^{(2)} = 0.070$	$p^{(2)} = 0.087$	$p^{(2)} = 0.087$	$p^{(2)} = 0.081$	

(% ± sd): mean mortality percentage ± standard deviation; (1): Friedman Test; (2): Kruskal Wallis Test.

The high larvicidal potential of *C. ferrea* was observed in *Ae. aegypti* Rockfeller strain, which presented an LC_{50} of 14.9 mg/mL, whereas *L. origanoides* showed better activity against *Ae. aegypti* Liverpool strain (LC_{50} = 15.9 mg/mL). For *Ae. aegypti* Recife population (wild strain), *C. ferrea* was the most promising plant with an LC_{50} of 16.5 mg/mL (Table 3).

Table 3. Larvicidal activity (LC_{50}) of *Caesalpinia ferrea* and *Lippia origanoides* against *Aedes aegypti* 48 h after exposure.

Extracts	*Ae. aegypti*	LC_{50} (CI) (mg / mL)
C. ferrea	Liverpool strain	22.2 (18.1 – 27.0)
	Rockfeller strain	14.9 (10.1 - 19.8)
	Recife population	16.5 (12.7 – 20.4)
L. origanoides	Liverpool strain	15.9 (9.7 – 21.4)
	Rockfeller strain	21.5 (14.8 – 28.9)
	Recife population	19.9 (12.8 - 27.1)

LC_{50}: mean lethal concentration; CI: confidence interval at 95% probability.

C. ferrea (78.8 mg/mL and 105 mg/mL) and *L. origanoides* (97.5 mg/mL and 130 mg/mL) caused midgut rupture and internal content extravasation of *Ae. aegypti* as observed in the microscopic examination. The anal gills were also damaged.

DISCUSSION

This study demonstrates high larvicidal activity against *Ae. aegypti* in compounds extracted from plants commonly found in the Northeastern region of Brazil. In general, the maximum activity for both plants was achieved within two hours using the highest concentration, except for *L. origanoides* in the Recife population, which occurred after six hours (Tables 1 and 2). Previous studies assessing the larvicidal potential of *C. ferrea* followed a similar trend to that observed here, but with a lower larvicidal potential (85%) (Cavalheiro et al., 2009). In fact, the larvicidal potential of *L. origanoides* against *Ae. aegypti* was demonstrated previously in other studies (Carvalho et al., 2003).

The variation of larvicidal activity noted in different studies may be related to several factors, such as the biotic conditions of culicids (e.g., instar larval and genetic features of strain/population), the variation of chemical compounds of different parts of plants (e.g., leaves, flowers, fruit, branches and roots), as well as abiotic factors related to the place of origin of the plant, such as temperature, humidity, rainfall and soil composition (Stashenko et al., 2010; Sampaio et al., 2016).

The chemical constitution of different plants, as known, indicates their potential activity. For instance, the chemical evaluation of *C. ferrea* showed the presence of compounds with insecticide action, such as flavonoids, saponins, tannins, coumarins, sterols and phenolic compounds (Cavalheiro et al., 2009). On the other hand, for *L. origanoides*, thymol and carvacrol are the main compounds with larvicidal activity (Carvalho et al., 2003; Cavalcanti et al., 2004; Silva et al., 2008).

Considering values of LC_{50}, *C. ferrea* presented better larvicidal potential against *Ae. aegypti* Rockfeller strain (LC_{50}= 14.9 mg/mL), while *L. origanoides* was more active against the Liverpool strain (LC_{50} = 15.9 mg/mL). For *Ae. aegypti* Recife population, *C. ferrea* was the most promising plant with a LC_{50} of 16.5 mg/mL (Table 3). A similar study assessing the larvicidal potential of *L. origanoides* against *Ae. aegypti* Rockfeller strain, found a LC_{50} of 40.1 ppm (Vera et al., 2014). The probable toxicity observed for the high concentrations used in this study should be histologically investigated, since the extravasation of the internal midgut content and the anal gills damage have also been observed in previous studies (Al-Mehmadi, 2011; Perumalsamy et al., 2013; Wang et al., 2019).

The constant use of chemical compounds to control insects over extended periods of time has induced the development of resistance (Macoris et al., 2014; Valle 2019). This phenomenon occurs worldwide, hampering the control of mosquitoes, the most important pathogen vectors of One Health relevance (Rodriguez et al., 2002). These chemical products used for mosquito control may cause damage to the environment due to lengthy residual effects, as well as being lethal to non-target organisms (Masetti, 2016; Wang et al., 2019). Nonetheless, there is an extensive search for alternatives to control these invertebrates using botanical insecticides (Benelli et al., 2018; Silva et al., 2008). In this sense, plants have been considered an important tool, mainly due to their greater selective, biodegradable, and less toxic characteristics in comparison to other chemical compounds (Silva et al., 2004; Pavela et al., 2019).

The findings in this study demonstrated that hydro ethanol extracts from *C. ferrea* and *L. origanoides* present larvicidal activity against *Ae. aegypti*. It is important to highlight that both plants have been well studied and several properties (antimicrobial, anti-inflammatory and, antiparasitic) have already been assessed. However, the larvicidal action of *C. ferrea* has not been sufficiently investigated. Finally, the data in this study reinforce the role of *L. origanoides* as a larvicidal agent and open new possibilities for the use of *C. ferrea* as a promising tool to control mosquitoes of medical and veterinary concern.

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