ORIGINAL ARTICLE

Larvicidal activity and chemical composition of essential oils against *Aedes aegypti* Linn (Diptera: Culicidae) and *Aedes albopictus Skuse* (Diptera: Culicidae)

Atividade larvicida e composição química de óleos essenciais contra *Aedes aegypti* Linn (Diptera: Culicidae) e *Aedes albopictus Skuse* (Diptera: Culicidae)

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Abstract

Introduction *Aedes* mosquitoes are the main vectors of the dengue, chikungunya, and Zika viruses. Essential oils have been used in research as alternatives to the synthetic insecticides traditionally used in programs to control these diseases. **Methods**: leaves of plants from the Caatinga biome were used to obtain essential oils which were used for larvicide essays against *Aedes aegypti* and *Aedes albopictus* larvae. **Results**: significant differences were observed in the LC_{50} values of *C. zeylanicum*, *C. winterianus*, *E. citriodora*, *O. micranthum*, and *T. erecta* essential oils in *Ae. aegypti*, while these differences were observed for *A. conyzoides*, *C. winterianus*, *E. citriodora*, and *O. micranthum* essential oils in *Ae. albopictus*, at the analyzed times. The LC_{50} for 24 h revealed a significant larvicidal effect for all tested samples, with emphasis on the effect of *A. conyzoides* and *L. gracilis* essential oils on *Ae. aegypti*, and the effect of *A. conyzoides*, *C. zeylanicum*, *C. winterianus*, *L. gracilis*, and *O. micranthum* essential oils on *Ae. albopictus*, all with an LC_{50} < 100 ppm. It was observed a predominance of terpenes as components in the essential oils, and, in some of them, representing the major constituent (citronellal: 47.71% in *E. citriodora* and 47.63% in *C. winterianus*; geraniol: 30.54% in *C. winterianus*; citronellol: 25.61% in *E. citriodora*; carvacrol: 55.13% in *L. gracilis*; and cyclohexen-1-one, 2-isopropyl-5-methyl-: 69.94% in *T. erecta*). The other major compounds observed were precocene (chromene - 97.66% - *A. conyzoides*), and eugenol (phenylpropanoid - 96.28% and 73.21% - *C. zeylanicum* and *O. micranthum*, respectively). **Conclusions:** the findings of this study revealed the larvicidal potential of these essential oils on *Ae. aegypti* and *Ae. albopictus* control, representing an alternative source to the traditional chemical controls used against the populations of these vectors. Further studies on the effects on non-target organisms and the combined action of two or more essential oils evaluated under field conditions are essential for obtaining commercially efficient formulations of these extracts.

Keywords: larvicide; essential oils; chemical composition; *Aedes aegypti* ; *Aedes albopictus*.

Resumo

Introdução: os mosquitos do gênero *Aedes* são os principais vetores dos vírus da dengue, chikungunya e Zika. Os óleos essenciais têm sido usados em pesquisas como alternativas aos inseticidas sintéticos tradicionalmente usados em programas de controle dessas doenças. **Métodos:** folhas de plantas do bioma Caatinga foram utilizadas para obter óleos essenciais que foram usados em ensaios larvicida contra *Aedes aegypti* e *Aedes albopictus*. **Resultados:** diferenças significativas foram observadas nos valores de CL_{50} dos óleos essenciais de *C. zeylanicum*, *C. winterianus*, *E. citriodora*, *O. micranthum* e *T. erecta* em *Ae. aegypti*, ao passo que diferenças significativas foram observadas para os óleos essenciais de *A. conyzoides*, *C. winterianus*, *E. citriodora* e *O. micranthum* em *Ae. albopictus*, nos tempos analisados. Os valores de CL_{50} para 24 h revelaram um efeito larvicida significativo para todas as amostras testadas, com destaque para os óleos essenciais de *A. conyzoides* e *L. gracilis* sobre *Ae. aegypti*, e para os óleos essenciais de *A. conyzoides*, *C. zeylanicum* e *L. gracilis* sobre *Ae. albopictus*, todos com uma CL_{50} < 100 ppm. Observou-se a predominância de terpenos na composição química dos óleos analisados, alguns dos quais representando o constituinte majoritário (citronelal: 47,71% em *E. citriodora* e 47,63% em *C. winterianus*; geraniol: 30,54% em *C. winterianus*; citronelol: 25,61% em *E. citriodora*; carvacrol: 55,13% em *L. gracilis*, e cyclohexen-1-one, 2-isopropyl-5-methyl-: 69,94% em *T. erecta*). Outros constituintes majoritários observados foram o precoceno (cromeno - 97,66% - *A. conyzoides*) e eugenol (fenilpropanoide – 96,28% e 73,21% - *C. zeylanicum* e *O. micranthum*, respectivamente). **Conclusões**: os resultados desse estudo revelaram o potencial larvicida desses óleos essenciais no controle de *Ae. aegypti* e *Ae. albopictus*, representando uma fonte alternativa ao controle químico tradicionalmente usado contra as populações desses vetores.

Palavras-chave: larvicida; óleos essenciais; composição química; *Aedes aegypti* ; *Aedes albopictus*.

INTRODUCTION

Aedes aegypti and *Aedes albopictus* are vectors of some important arboviruses in the global public health scenario, including dengue, chikungunya, and Zika,[1] causing extensive morbidity and mortality, in addition to representing a great

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economic burden in endemic countries[2]. Climate change, the disorderly occupation of urban areas, and poor basic sanitation are factors that favor viral amplification and dissemination of these diseases[3].

Although Brazil has a vaccine against dengue viruses provided by the public health system, its large-scale supply is not yet available, which prevents its use as collective protection in the short term. Furthermore, because of the absence of specific treatments for these arboviruses, combating their insect vectors has been the most efficient way to reduce the transmission of these pathogens to human populations since there are no vaccines against chikungunya and zika viruses. Conventionally, these actions aim to reduce the number of breeding sites that harbor their immature forms and reduce the density of their populations (immature and adult) through chemical, mechanical, or biological methods[4,5]. Synthetic insecticides, such as organochlorine and organophosphate, have been widely used for this purpose despite their high cost, role in selecting resistant populations, high toxicity to humans and other non-target organisms, and high contamination potential of soil and water[6]. In view of the problems arising from the systematic use of synthetic insecticides, new alternatives have been sought to control insect vectors of pathogens. Natural products of plant origin, such as plant extracts and essential oils, are among the most promising[7].

Essential oils are complex mixtures of secondary metabolites from aromatic plants with high insecticidal and repellent potential. When compared to synthetic insecticides, essential oils, in general, have some advantages, such as their biodegradability, the fact that they are species-specific with minimal side effects on non-target organisms, and the fact that they reduce the rates of resistance development in insect-target populations because of the large amount and diversity of substances present in their composition[8,9].

This study aimed to evaluate the larvicidal effects of essential oils obtained from seven Brazilian plants on *Ae. aegypti* and *Ae. albopictus* and identify their constituents.

METHODS

a) Plant material

Leaves of Ageratum conyzoides L. (Asteraceae), Cinnamomum zeylanicum Blume (Lauraceae), Cymbopogon winterianus Jowitt ex Bor (Poaceae), Eucalyptus citriodora Hook (Myrtaceae), Lippia gracilis Schauer (Verbenaceae), Ocimum micranthum Willd (Lamiaceae), and Tagetes erecta L. (Asteraceae) were collected at Horto de Plantas Medicinais of the Federal University of Ceará.

b) Essential oil extraction and chemical characterization

Newly collected leaves were macerated and immediately weighed. These materials were placed in round-bottom flasks (5 L), followed by extracting the respective essential oils through hydrodistillation in a Clevenger-type device[10]. The essential oils obtained were treated with anhydrous sodium sulfate (Na_2SO_4) to remove any traces of water or moisture and then stored in amber glass containers (10 mL) at 4ºC.

Analysis of the essential oil constituents was performed using gas chromatography coupled with mass spectrometry (GC/MS)[11]. The equipment used was an Agilent 7890B gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled with a quadrupole mass detector (model 5977A; Agilent). The samples, diluted 1:100 with hexane (HPLC grade, Merck), were injected directly into a CombiPAL autosampler (CTC Analytics, Switzerland), with the injector temperature equal to 250°C. The separation of compounds was carried out on an HP5-MS capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) made of fused silica (Restek Co., Bellefonte, PA, USA). The oven temperature was programmed to increase from 70°C to 180 °C at 4°C/min, from 180 °C to 250°C at 10°C/min, and then maintained at 250°C for 5 minutes. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. The interface temperature was maintained at 250ºC. Ionization was performed using electron ionization (+70 eV) mode, with the ion source maintained at 150°C and scanning from 35 to 350 m/z.

Substance identification was performed by comparing the obtained mass spectra with those provided by the National Institute of Standards and Technology (NIST 1.6 MS Library, Gaithersburg, MD, USA). Furthermore, the experimental retention index (RI) was determined using a homologous mixture of n-alkanes (C9-C30) and compared with those reported in the literature and online databases (NIST Chemistry Webbook, Chemspider, and Pubchem).

c) *Aedes aegypti* and *Aedes albopictus* larvae and larvicidal assays

The third- and fourth-instar larvae of *Ae. aegypti* and *Ae. albopictus* from different neighborhoods in the city of Fortaleza were used for larvicide tests with the essential oils obtained. The larvae were kept in different colonies, according to the species, at the Laboratory of Vectors, Reservoirs, and Venomous Animals, Dr. Thomás Corrêa Aragão, from the Secretary of Health of the State of Ceará, and maintained under controlled conditions of temperature (25 ± o C), relative humidity (80 ± 10%), light and dark photoperiod (12:12), and fed with fish meal until the beginning of the tests. Once the colonies were established, they were maintained for up to three generations, at the end of which new colonies were started for further testing.

The tests were performed according to the methodology described by the World Health Organization[12] and adapted to the requirements of this research. For each test, different amounts of essential oils were diluted in 0.5 mL of dimethyl sulfoxide (DMSO) and then distilled water was added to complete a volume of 20 mL of solution (2.5% v/v). Twenty larvae were placed in glass beakers containing different concentrations of an aqueous suspension of the essential oil being tested. Three

replicates were performed simultaneously for each essential oil concentration, with a 2.5% DMSO solution (v/v) as control. The larvae were subjected to treatment at an initial concentration of 1000 mg/L to verify whether these extracts could cause the death of exposed larvae 24 hours after the beginning of the tests. Once a mortality rate of 60% or more was observed, lower concentrations of these products were used to determine the concentration that could kill 50% of the larvae (LC_{50}) after 24 and 48 hours of exposure to the natural products.

d) Data analysis

Data analysis regarding larvicidal activity and determination of LC_{50} was performed using the dose-response analysis method[13].

RESULTS AND DISCUSSION

The larvicidal activities of the essential oils tested against *Ae. aegypti* and *Ae. albopictus*, observed 24 and 48 h after the start of the tests, and measured from the values obtained from the LC_{50} at each time point, are shown in Table 1. Significant differences were observed in the LC_{50} values of Cinnamomum zeylanicum, Cymbopogon winterianus, Eucalyptus citriodora, Ocimum micranthum, and Tagetes erecta essential oils in *Ae. aegypti* at the analyzed times. In contrast, these differences were observed for Ageratum conyzoides, Cymbopogon winterianus, Eucalyptus citriodora, and Ocimum micranthum essential oils in *Ae. albopictus*. There was no significant difference between the LC_{50} of Cinnamomum zeylanicum and Tagetes erecta essential oils on *Ae. albopictus*. It was also observed that Ageratum conyzoides essential oil killed 100% of the larvae in *Ae. aegypti* at 48 h, the same effect was observed for Lippia gracilis essential oil on *Ae. aegypti* and *Ae. albopictus* (Table 1). The maintenance or even an increase in the larvicidal effect of a compound or substance over time (residual effect) is an important factor in the choice of larvicidal candidates[12].

Table 1. Larvicidal activity of essential oils obtained from different plant species on *Ae. aegypti* and *Ae. albopictus*, with the respective LC_{50} (ppm), after 24 and 48 h.

Plant species	*Aedes aegypti*		*Aedes albopictus*	
	LC_{50} ‡ CI ppm (24 h)	**LC_{50} ‡ CI ppm (48 h)**	**LC_{50} ‡ CI ppm (24 h)**	**LC_{50} ‡ CI ppm (48h)**
Ageratum conyzoides	41.614 (37.060 – 46.169)^{a}	-	69.796 (67.459 – 72.133)^{Ab}	59.864 (57.121 – 62.607)^{B}
Cinnamomum zeylanicum	104.116 (86.074 – 122.158)^{Aa}	75.515 (70.889 – 80.141)^{Ba}	78.333 (73.864 – 82.803)^{Ab}	72.301 (67.985 – 76.616)^{Aa}
Cymbopogon winterianus	110.563 (106,972 – 114.154)^{Aa}	78.983 (72.628 – 85.338)^{Ba}	99.627 (97.145 – 102.109)^{Ab}	92.078 (89.092 – 95.063)^{Bb}
Eucalyptus citriodora	181.420 (174.219 – 188.621)^{Aa}	159.411 (152.579 – 166.243)^{Ba}	131.938 (119.380 – 144.496)^{Ab}	97.318 (73.427 – 121.210)^{Bb}
Lippia gracilis	28.825 (23.817 – 33.833)^{a}	-	40.515 (35.031 – 45.999)^{b}	-
Ocimum micranthum	165.721 (134.795 – 196.647)^{Aa}	63.821 (56.570 – 71.072)^{Ba}	99.458 (89.630 – 109.286)^{Ab}	82.601 (71.667 – 93.534)^{Bb}
Tagetes erecta	116.278 (100.68 – 131.875)^{Aa}	67.229 (57.883 – 76.574)^{Ba}	255.398 (212.968 – 297.828)^{Ab}	231.248 (198.751 – 263.745)^{Ab}

* Capital letters represent comparisons at 24 and 48 h for each plant and mosquito species combination. Lowercase letters compare the mosquito species at each time point. (-) Mortality of all larvae.

† LC_{50} = lethal concentration (ppm) at which 50% were killed.

‡ CI = confidence interval, 95%.

The LC_{50} values of the essential oils used in this study obtained 24 h after the beginning of the tests, revealed a significant larvicidal effect for all tested samples[14] with emphasis on the effect of Ageratum conyzoides and Lippia gracilis essential oils on *Ae. aegypti* and the effect of Ageratum conyzoides, Cinnamomum zeylanicum, and Lippia gracilis essential oils on *Ae. albopictus*, all with an LC_{50} < 100 ppm (Table 1). These values (LC_{50} – 24 h) also indicated that, except for Ageratum conyzoides and Lippia gracilis essential oils, *Ae. albopictus* had a higher susceptibility to the essential oils used than *Ae. aegypti* (Table 1). These differences may be related to intra-and interspecific differences that naturally occur in these mosquito populations,

which are subject to different environmental selection pressures, thus influencing their resistance/susceptibility[15].

In addition, it is important to emphasize that the chemical composition of essential oils in a plant species varies according to the plant organ and developmental stage and is strongly determined by genetic (intrinsic) and environmental (extrinsic) factors[16], resulting in diverse plant chemotypes[17]. Significant differences were observed in the composition of Tagetes erecta essential oils between this study and those reported by Laosinwattana et al. (2018)[18]. Such differences reflect a greater or lesser biological effect, such as the smaller larvicidal effect of Tagetes erecta essential oil on *Ae. aegypti* in 24 hours observed in this study (LC_{50} = 116.27 ppm) compared with that observed by Marques et al. (2011) (LC_{50} = 79.78 ppm)[19]. It explains why specimens of the same plant species from different geographic regions exhibit different larvicidal effects against *Ae. aegypti* and *Ae. albopictus*[20,21,22,23,24,25].

Concerning chemical composition of the essential oils used in this study, a predominance of terpenes was observed. For some essential oils (citronellal: 47.71% in Eucalyptus citriodora and 47.63% in Cymbopogon winterianus; geraniol: 30.54% in Cymbopogon winterianus; citronellol: 25.61% in Eucalyptus citriodora; carvacrol: 55.13% in Lippia gracilis; and cyclohexen-1-one, 2-isopropyl-5-methyl-: 69.94% in Tagetes erecta), it was the major compound(s) (20–70%) of the essential oil. The other major compounds observed were precocene (chromene), constituting 97.66% of the essential oil from Ageratum conyzoides, and eugenol (phenylpropanoid), constituting 96.28% and 73.21% of the essential oils from Cinnamomum zeylanicum and Ocimum micranthum, respectively (Table 2). Figure 1 shows the molecular structures of these substances.

Table 2. Constituents of essential oils.

Plant species	Compound name	Class of compounds	RT (min)	Percentage of total composition	RIexp	Main fragment ions
Ageratum conyzoides	Caryophyllene	Terpene	16.903	1.93	1422	41, 69, 79, 93, 105, 120, 133 (BP),147, 161, 189, 204 (M+●)
	Humulene	Terpene	17.928	0.17	1456	41, 67, 80, 93 (BP), 121,147, 204 (M+●)
	Precocene	Chromene	18.252	97.66	1467	132, 160,175 (BP), 190 (M+●)
	β-Sesquiphellandrene	Terpene	19.995	0.25	1526	27, 41, 69 (BP), 93, 120, 133,161, 204 (M+●)
	3-Carene	Terpene	5.763	0.08	1032	41, 67, 79, 93 (BP), 105, 121, 136 (M+●)
	Linalol	Terpene	7.373	1.44	1101	27, 41,43, 55, 69, 71 (BP), 80, 93, 105, 121, 136 (M+●)
	α-Terpineol	Terpene	10.071	0.06	1197	41, 43, 59 (BP), 67, 81, 93, 107, 121, 136, 154 (M+●)
Cinnamomum zeylanicum	Eugenol	Phenylpropanoid	15.071	96,28	1362	39, 55, 77, 91, 103, 131, 149, 164 (M+●)
	Humulene	Terpene	17.934	0.11	1456	41, 67, 80, 93 (BP), 121,147, 204 (M+●)
	□-Elemene	Terpene	19.207	0.06	1499	41,55, 67, 79, 81, 91, 93, 107, 121 (BP), 136, 147, 161,189, 204 (M+●)
	Eugenol acetate	Phenylpropanoid	20.11	0.91	1530	43, 77, 91, 133, 149,164 (BP), 206 (M+●)
	Spathulenol	Tricyclic sesquiterpene alcohol	21.56	0.09	1580	41, 43 (BP), 69, 79, 91, 105, 119, 159, 162, 177, 187, 202, 205, 220 (M+●)
	Caryophyllene oxide	Terpene	21.713	0.96	1585	27, 39, 41, 43 (BP), 55, 69, 79, 91, 93, 109, 121, 149, 161, 177, 187, 205, 220 (M+●)
	Humulene epoxide I	Terpene	22.464	0.09	1612	39, 41, 43, 55, 67, 81, 93, 96, 109 (BP), 123, 138, 147, 220 (M+●)

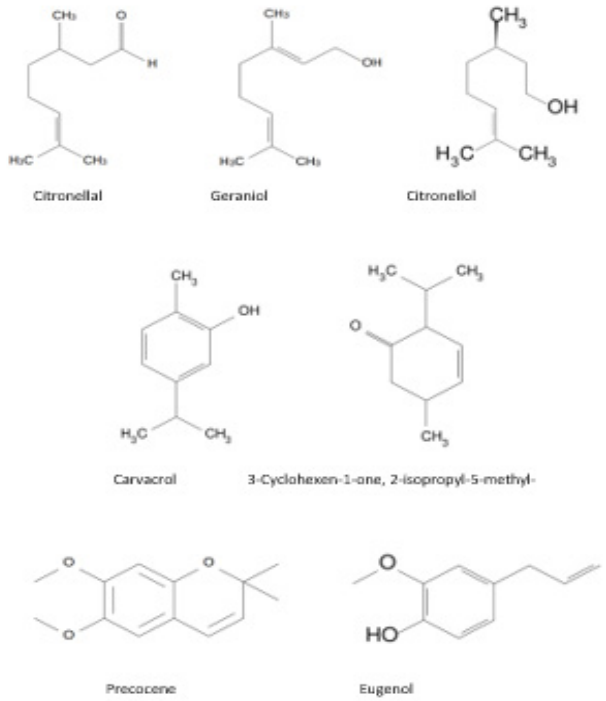
Plant species	Compound name	Class of compounds	RT (min)	Percentage of total composition	RIexp	Main fragment ions
	D-Limonene	Terpene	5.725	1.28	1030	41, 53, 68 (BP), 93, 107, 121, 136 (M+●)
	Isopulegol	Terpene	8.683	0.72	1148	27, 39, 41 (BP), 55, 69, 71, 81, 84, 95, 111, 121, 136, 139, 154 (M+●)
	Citronellal	Terpene	8.842	47.63	1153	27, 29, 39, 41 (BP), 55, 69, 84, 95, 111, 121, 136, 139, 154 (M+●)
	Citronellol	Terpene	11.006	11.79	1228	27, 29, 31, 39, 41, 55, 67, 69 (BP), 81, 82, 95, 109, 123, 138, 156 (M+●)
Cymbopogon winterianus	Geraniol	Terpenic alcohol	11.820	30.54	1255	27, 29, 39, 41, 55, 68, 69 (BP), 84, 93, 121, 123, 139, 154 (M+●)
	Geranyl acetate	Terpene	15.796	1.67	1385	41, 43, 53, 68, 69 (BP), 80, 93, 107, 121, 136, 154, 196 (M+●)
	-Cadinene	Terpene	19.982	0.86	1525	29, 41, 43, 55, 67, 77, 81, 91, 105, 119, 133, 147, 161 (BP), 189, 204 (M+●)
	Elemol	Terpene	20.727	5.51	1551	29, 41, 43, 59 (BP), 69, 81, 93, 107, 121, 135, 149, 161, 175, 189, 204, 222 (M+●)
Eucalyptus citriodora	β-Pinene	Terpene	4.714	0.23	981	27, 39, 41, 53, 69, 77, 79, 91, 93 (BP), 107, 121, 136 (M+●)
	Eucalyptol	Terpene	5.795	0.36	1033	27, 39, 41, 43 (BP), 55, 69, 71, 81, 84, 93, 96, 108, 111, 125, 136, 139, 154 (M+●)
	Rose oxide L	Terpene	7.710	0.23	1113	27, 29, 39, 41, 55, 69, 83, 139 (BP), 154 (M+●)
	Isopulegol	Terpene	8.665	16.40	1147	27, 39, 41 (BP), 55, 69, 71, 81, 84, 95, 111, 121, 136, 139, 154 (M+●)
	Citronellal	Terpene	8.849	47.71	1154	27, 29, 39, 41 (BP), 55, 69, 84, 95, 111, 121, 136, 139, 154 (M+●)
	Citronellol	Terpene	11.012	25.61	1228	27, 29, 31, 39, 41, 55, 67, 69 (BP), 81, 82, 95, 109, 123, 138, 156 (M+●)
	Citronellol acetate	Terpene	14.829	9.27	1354	27, 29, 31, 39, 41, 43 (BP), 55, 67, 69, 81, 82, 95, 109, 123, 138, 198 (M+●)
	Caryophyllene	Terpene	16.903	0.19	1422	41, 69, 79, 93, 105, 120, 133 (BP),147, 161, 189, 204 (M+●)
Lippia gracilis	β-Thujene	Terpene	3.842	0.67	928	27, 39, 41, 53, 65, 77, 79, 91, 92, 93 (BP), 121, 136 (M+●)

Plant species	Compound name	Class of compounds	RT (min)	Percentage of total composition	RIexp	Main fragment ions
	β-Pinene	Terpene	4.885	1.25	992	27, 39, 41, 53, 69, 77, 79, 91, 93 (BP), 107, 121, 136 (M+●)
	o-Cymene	Hydrocarbon (Aromatic)	5.623	11.55	1026	27, 39, 51, 65, 77, 91, 117, 119 (BP), 134 (M+●)
	Eucalyptol	Terpene	5.789	1.38	1033	27, 39, 41, 43 (BP), 55, 69, 71, 81, 84, 93, 96, 108, 111, 125, 136, 139, 154 (M+●)
	γ-Terpinene	Terpene	6.393	5.87	1059	27, 39, 41, 43, 77, 79, 84, 91, 93 (BP), 105, 119, 121, 136 (M+●)
	Thymol methyl ether	Terpene	11.222	4.25	1235	15, 39, 51, 65, 77, 91, 105, 117, 119, 134,149 (BP), 164 (M+●)
	Thymol	Phenol	12.946	4.49	1292	39, 51, 65, 77, 91, 117, 135 (BP), 150 (M+●)
	Carvacrol	Terpene	13.277	55.13	1303	77, 91, 117, 135 (BP), 150 (M+●)
	Caryophyllene	Terpene	16.910	5.76	1422	41, 69, 79, 93, 105, 120, 133 (BP),147, 161, 189, 204 (M+●)
	(-)-cis-beta-Elemene	Terpene	19.206	7.98	1499	41,55, 67, 79, 81, 91, 93, 107, 121 (BP), 136, 147, 161,189, 204 (M+●)
	(-)-Spathulenol	Alcohol	21.548	1.67	1580	41, 43(BP), 55, 69, 79, 91, 93, 105, 119, 131, 147, 159,187, 202, 205, 220 (M+●)
Ocimum micranthum	Eucalyptol	Terpene	5.789	2.64	1033	27, 39, 41, 43 (BP), 55, 69, 71, 81, 84, 93, 96, 108, 111, 125, 136, 139, 154 (M+●)
	Eugenol	Phenylpropanoid	15.071	73.21	1362	39, 55, 77, 91, 103, 131, 149, 164 (M+●)
	(-)-cis-beta-Elemene	Terpene	16.070	3.86	1394	41,55, 67, 79, 81, 91, 93, 107, 121 (BP), 136, 147, 161,189, 204 (M+●)
	Caryophyllene	Terpene	16.916	9.46	1422	41, 69, 79, 93, 105, 120, 133 (BP),147, 161, 189, 204 (M+●)
	Humulene	Terpene	17.921	1.77	1456	41, 67, 80, 93 (BP), 121,147, 204 (M+●)
	β-Selinene	Terpene	18.901	1.28	1488	27, 29, 39, 41 (BP), 55, 67, 81, 91, 93, 105, 107, 121, 133, 147, 161, 175,189, 204 (M+●)
	(-)-cis-beta-Elemene	Terpene	19.206	5.29	1499	41,55, 67, 79, 81, 91, 93, 107, 121 (BP), 136, 147, 161,189, 204 (M+●)
	β-Bisabolene	Terpene	19.461	0.98	1510	41, 55, 67, 69 (BP), 79, 93, 109, 119, 121, 135, 147, 161,189, 204 (M+●)

Plant species	Compound name	Class of compounds	RT (min)	Percentage of total composition	RIexp	Main fragment ions
	Elemicin	Organic compound (phenylpropene)	20.931	1.51	1558	41, 77, 91, 133, 150, 165, 177,193, 208 (M+●; BP)
Tagetes erecta	D-Limonene	Terpene	5.719	7.55	1030	41, 53, 68 (BP), 93, 107, 121, 136 (M+●)
	trans-β-Ocimene	Terpene	5.859	1.15	1036	41, 53, 79, 93 (BP), 105, 121, 136 (M+●)
	β-Ocimene	Terpene	6.101	7.02	1046	27, 41, 53, 79, 93 (BP), 105, 121, 136 (M+●)
	α- Terpinolen	Terpene	7.131	7.43	1091	27, 39, 41, 53, 65, 77, 79, 91, 93 (BP), 105, 121, 136 (M+●)
	3-Cyclohexen-1-one, 2-isopropyl-5-methyl-	Terpene	11.89	69.94	1251	27, 39, 41, 54, 82, 95, 109, 110 (BP), 137, 152 (M+●)
	Caryophyllene	Terpene	16.903	2.26	1422	41, 69, 79, 93, 105, 120, 133 (BP),147, 161, 189, 204 (M+●)

* RT: Retention time (min)
† RIexp: Experimental Retention Index in column HP-5.
‡ RIlit: Literarture Retention Index obtained from Chemistry WebBook NIST for HP-5 column

Figure 1. Molecular structures of the major essential oil components.



Because of the volatility of their aromatic components, essential oils impart a distinctive odor, flavor, or aroma to the plants that produce them. In nature, these volatile secondary metabolites act as pollinator attraction factors, protect plants against cold and heat, and form part of the defense against pests and microorganisms[9].

Essential oils are widely used as bactericidal, virucidal, fungicidal, antiparasitic, insecticidal, and cosmetic agents.[8,26] Recently, essential oils have been the subject of investigation for their larvicidal effect on many species of insect vectors of human pathogens, representing alternative and sustainable sources for chemical control currently in use for these insects. As they are complex mixtures containing varying amounts of a wide range of substances, the biological properties of essential oils may be directly related to their major components[27] and/or to the synergistic effect of two or more of their components[9].

Therefore, the larvicidal activity of Ageratum conyzoides essential oil on *Ae. aegypti* and *Ae. albopictus* in this study was strongly associated with precocene, which inhibits the synthesis of juvenile hormones in insects.[28] Comparative analysis of the LC_{50} of Ageratum conyzoides essential oil and its major constituents (precocene I and II) on *Ae. albopictus*, demonstrated a significant larvicidal effect of these constituents (LC_{50} precocene I = 43.55 ppm, and LC_{50} precocene II = 41.6 ppm) in relation to the essential oil as a whole (LC_{50} = 61.22 ppm)[21].

Eugenol, commonly obtained from the essential oils of plants belonging to the Lamiaceae, Lauraceae, Myrtaceae, and Myristicaceae families,[29] was identified as the major constituent of Cinnamomum zeylanicum (96.28%) and Ocimum micranthum (73.21%) essential oils in this study. This compound has a strong larvicidal effect against *Ae. aegypti*,[30,31] in addition to exerting pronounced insecticidal effects on a wide range of domestic arthropods.[32] However, owing to its low chemical stability, high sensitivity to oxidation, and various chemical interactions, it is subject to structural changes, which significantly increase its insecticidal properties[33].

The larvicidal effect of an essential oil or its components is directly related to its structural (presence of double bonds, aromatic rings, low number of hydroxyl groups, and acetylation of hydroxyl groups in oxygenated monoterpenes) and physicochemical (lipophilicity) characteristics[34,35]. Carvacrol, citronellal, and citronellol, major constituents of Lippia gracilis (55.13%), Cymbopongon winterianus (47.63%), Eucalyptus citriodora (47.71%), and Eucalyptus citriodora (25.61%) essential oils in this study, exhibited a strong inhibitory effect on the enzyme acetylcholinesterase (AChE), a fact that has been associated with its high larvicidal effect on *Ae. aegypti* and *Ae. albopictus*[39], since AChE is widely recognized as the target of organophosphate and carbamate insecticides[36,37,38]. Geraniol, the major constituent of Cymbopogon winterianus essential oil (30.54%) in this study, also exhibited a strong larvicidal effect on these Culicidae species;[39] this effect is associated with GABA receptors whose activation leads to inhibition of neurotransmission[40].

CONCLUSIONS

The findings of this study revealed the larvicidal potential of essential oils obtained from the leaves of Ageratum conyzoides, Cinnamomum zeylanicum, Cymbopogon winterianus, Eucalyptus citriodora, Lippia gracilis, Ocimum micranthum, and Tagetes erecta on *Ae. aegypti* and *Ae. albopictus*, thus representing an alternative source to the traditional chemical controls used against the populations of these vectors. Further studies on the effects of essential oils in insect development, on non-target organisms, combining the action of two or more essential oils, evaluated under field conditions and addressed to different insect populations, are essential for obtaining commercially efficient formulations of these extracts.

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