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Article

Tetradenia riparia leaves, flower buds, and stem essential oils to control of Aedes *aegypti* larvae

Giuliana Zardeto[1], Renan Almeida de Jesus[1], Herika Line Marko de Oliveira[1], José Eduardo Gonçalves[2,3], Ranulfo Piau Junior[4], Ezilda Jacomassi[5], Zilda Cristiani Gazim[1*]

[1]Post graduate Program in Biotechnology Applied to Agriculture, Paranaense University (UNIPAR), Umuarama, Paraná, Brazil, [2]Post graduate Program in Clean Technologies and [3]Cesumar Institute of Science, Technology and Innovation – ICETI, UniCesumar, Maringá, Paraná, Brazil, [4]Post graduate Program of Animal Science, Paranaense University (UNIPAR), Umuarama-Paraná, Brazil, [5]Professional Master's Graduate Program in Medicinal and Phytotherapeutic Plants in Primary Care. Paranaense University (UNIPAR), Umuarama-Paraná, Brazil

Tetradenia riparia (Hochst.) Codd (Lamiaceae) is a species native to the African continent and used as an insect repellent. The objective of the study was to evaluate the larvicidal potential of essential oils (EOs) from the leaves, flower buds, and stem of *T. riparia,* collected in winter against *Aedes aegypti* larvae. The EOs were extracted by hydrodistillation (3 h) and identified by GC/MS. The EOs were tested against larvae of *A. aegypti* at concentrations ranging from 12500 to 1.5 µg/mL for 24 h. The insecticide activity was evaluated by probit analysis, and the anticholinesterase activity was determined by bioautographic method. The results of the class projection indicated sesquiterpenes as the majority class, corresponding to 60.66% (leaves), 64.70% (flower buds) and 83.99% (stem), and the bioassays on *A. aegypti* larvae indicated LC_{50} of 1590, 675 and 665 µg/mL, respectively. The anticholinesterase activity indicated that the EO of the leaves inhibited the enzyme at a concentration of 780 µg/mL, and those from the flower buds and stem inhibited up to 1560 µg/mL. The results indicated weak activity of essential oils against *A. aegypti* larvae.

Keywords: Sesquiterpenes. Larvicide. 14-hydroxy-9-epi-caryophyllene. Fenchone. α-cadinol.

INTRODUCTION

The *Aedes aegypti* vector is responsible for transmitting yellow fever, dengue, severe dengue, zika, and chikungunya, leading to human mortality in many countries (OMS, 2019). In Brazil, in 2019, 1. 544. 987 dengue cases were recorded with 782 deaths, besides 132. 205 cases of chikungunya with 92 deaths, and 10. 768 cases of Zika virus with three deaths (Brasil, 2020).

To circumvent this problem, it is essential to identify new larvicides with different mechanisms of action to increase the options available to use them as means of control in public health. The ideal larvicide must be effective, ecologically correct, sustainable, profitable, and have low toxicity in mammals (Gois *et al*., 2013).

To achieve effective measures to control this mosquito, it is necessary to consider problems such as unplanned urbanization, poor sanitary conditions, and inadequate water supply (Gupta, Reddy, 2013). Thus, in the infected areas, strategies are carried out to eliminate mosquito outbreaks, such as chemical and biological control and environmental management (Braga, Valle, 2007).

Chemical control is performed according to the development phase of the vector. In the larval stage of *A. aegypti*, larvicidal substances are used, mainly organophosphates, such as malathion, and juvenile hormone analogues, such as pyriproxyfen (Braga, Valle, 2007; Brasil, 2020), which are applied in places with water

***Correspondence:** Z. C. Gazim. Programa de Pós Graduação em Biotecnologia Aplicada à Agricultura. Universidade Paranaense (UNIPAR). Praça Mascarenhas de Moraes, 4282. CEP 87502-210, Umuarama, Paraná, Brazil. E-mail: cristianigazim@prof.unipar.br*. Orcid: https://orcid.org/0000-0003-0392-5976

deposits. In adulthood, insecticides are used in the form of sprinklers to reduce vector density (Braga, Valle, 2007). However, the use of these synthetic insecticides must be judicious to delay the development of resistance (Zara *et al*., 2016), toxicity to humans, and the impact they cause on biodiversity (Zara *et al*., 2016). The use of plants as insect repellents is common, as shown by Kweka *et al*., (2008). Thus, it is necessary to investigate the potential of plants for insect control (Kweka *et al*., 2008).

In this sense, research has been carried out using essential oils (EOs) extracted from plants such as parsley (*Petroselinum crispum*) (Camilotti *et al*., 2015) and citronella (*Cymbopogon nardus and Cymbopogon winterianum*) (Castro, Ramos, 2013) since the structural and chemicals groupings of these essential oils or the combination between them can provide and intensify the larvicidal potential (Fernandez *et al*., 2018).

In this context, our research group investigated the larvicidal potential of *Tetradenia riparia* (Lamiaceae), known as false myrrh (Souza, Lorenzi, 2012). It is a plant used in folk medicine and whose essential oil extracted from leaves has been used for the treatment of malaria, cryptococcosis, candidiasis, and respiratory infections (Okem, Finnie, Van Staden, 2012). Research carried out with this species indicates antimicrobial activity (Gazim *et al*., 2010), repellent activity against *Anopheles gambiae* (Omolo *et al*., 2004), acaricide activity against *Rhipicephalus (Boophilus) microplus* (Gazim *et al*., 2011) and antileishmanial activity (Demarchi *et al*., 2015). Hence, the present study aims to characterize the chemical composition and to evaluate the larvicidal potential of the essential oil of the leaves, flower buds, and stem of *T. riparia* against *A. aegypti*.

MATERIAL E METHODS

Plant material and essential oil extraction

T. riparia leaves, flower buds, and stem were collected in July 2017, (between 8 and 10 a.m), at latitude 23º46'16''S and longitude 53º19'38''W, and altitude of 442 m. The collection period coincided with the vegetative period of myrrh, that is, with the appearance of flower buds, during winter. A sample was authenticated and deposited at the Herbarium of Universidade Paranaense, UNIPAR, under the number 2502. This species is registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration number AA6C8A8.

Fresh samples of leaves, flower buds, and stem of *T. riparia* (150 g) were used. The extraction of the essential oil was performed by hydrodistillation, using modified Clevenger apparatus for 3 hours (Gazim *et al*., 2014). After the distillation, the essential oil was stored in an amber flask at -4 °C.

Chemical composition

The chemical identification of the essential oil components was performed by GC-MS (Agilent 7890B - 5977A MSD). The capillary column was HP-5MS UI 5% (30 mx 0.25 mm x 0.25 μm), with an initial temperature of 80 ° C (1 min) to 185 ° C (2 ° C min, remaining for 1 min), followed by heating of 9 ºC / min until reaching the temperature of 275 ° C, remaining for 2 min and ending with heating of 25 ° C / min up to 300 ° C, remaining for 1 min. Helium was used as carrier gas at a linear velocity of 1 mL min-1 to 300 ° C and a pressure release of 8.23 psi. The injector temperature was 280 ° C and the injection volume was 2 μL; the injection occurred in Split mode (20: 1), with the injector temperature maintained at 220 ° C. The transfer line was maintained at 280 °C, the ionization source and quadrupole at 230 ° C and 150 °C, respectively. The MS detection system was used in the "scan" mode, at the mass rate/charge rate/charge (m/z) of 40-550, with a "solvent delay" of 3 min. The compounds were identified by comparing the mass spectra found in NIST 11.0 libraries and the retention indices (RI), obtained by a homologous series of standard (C7-C28) (Adams, 2017).

Analysis of major compounds

For each sample of essential oil, the main chemical compounds identified and their respective chemical classes, as well as the amount, were transformed by the principal component analysis (PCA) in orthogonal latent variables, named major compounds, which are linear combinations of original variables created with

the eigenvalue of the data covariance matrix (Hair *et al*., 2005). Kaiser's criterion was used to choose the main compounds in which an eigenvalue preserves relevant information when it is superior to the unit (Camacho, Picó, Ferrer, 2010). The analysis was presented in graphic form (biplot) using Statistica 13.3 software (Statsoft, 2017).

Activity of *Tetradenia riparia* essential oil on *Aedes aegypti* larvae

For the bioassays, larvae from the third stage of *A. aegypti* were used, originating from the Vector Transmitted Endemic Control Center of the Secretariat of Sanitary Surveillance in the municipality of Cianorte, Paraná. The EOs of the leaves, flower buds, and stem were diluted in 2% aqueous polysorbate (80) solution at concentrations of 12500; 6250; 3120; 1560; 780; 390; 195; 97.5; 48.75; 24.37; 12.18; 6.09; 3.04 and 1.52 µg/mL. Ten larvae of *A. aegypti* were placed in 250 mL flasks with 10 mL of different concentrations of EOs. For the negative control, a 2% aqueous polysorbate (80) solution was used; the positive control was an organophosphate based on Temephós® at a concentration of 400000 µg/mL (Camargo *et al.,* 1998). The larvae were exposed to EO for 24 h, and those that showed no movement and did not respond to stimuli were considered dead (Cavalca *et al*., 2010).

Anticholinesterase activity of essential oil

The anticholinesterase activity was determined by the bioautography method described by Marston *et al*. (2002) with modifications (Yang *et al*., 2009). *T. riparia* EOs were tested starting from a concentration of 50.000 to 780 µg/mL and diluted in methanol. The samples were plotted on aluminum chromatoplates (10 x 10 cm, silica gel 60 F254 0.2 mm thick); after the plot, the plates were dried and a solution of enzyme acetylcholinesterase was sprayed on them in a buffer solution; then, the α-naphthyl acetate solution was sprayed.

The plates were kept at 37 ° C for 20 min. After this period, the chromatoplates were sprayed with Fast Blue B salt colorimetric reagent, resulting in a purple surface. As a positive standard, Temephós® larvicide was used.

Statistical analysis

The experiment had a completely random design. The data were submitted to analysis of variance (ANOVA), and the differences among the arithmetical averages were determined by Tukey's test at 5% significance (IBM SPSS Statistics 20). The lethal concentration (LC) that killed 50% (LC_{50}) and 99% (LC_{99}) of adult ticks and larvae and the respective confidence interval (CI) ($\alpha = 0.05$) were calculated by Probit analysis (ED 50 Plus version 1.0).

RESULTS

Through chemical analysis by GC / MS (Table I), the compounds present in the EO of the leaves, flower buds, and stem of *T. riparia* were identified.

TABLE I - Chemical composition of *Tetradenia riparia* leaves, flower buds and stem essential oil

Peak	[a]Compounds	RI Calc	Relative area (%)			Methods of Identification
			Leaves	Flower buds	Stem	
1	*α*-pinene	900	1.57	0.23	1.59	a,b,c
2	Camphene	911	1.22	0.16	1.59	a,b,c
3	Sabinene	926	1.41	0.34	0.06	a,b,c
4	*β*-pinene	930	0.73	0.23	0.98	a,b,c

TABLE I - Chemical composition of *Tetradenia riparia* leaves, flower buds and stem essential oil

Peak	[a]Compounds	RI Calc	Relative area (%)			Methods of Identification
			Leaves	Flower buds	Stem	
5	Limonene	1031	1.16	0.37	0.26	a,b,c
6	L-fenchone	1108	11.57	6.01	0.39	a,b,c
7	Fenchol	1116	0.81	0.55	0.07	a,b,c
8	L-camphor	1128	2.38	1.36	0.09	a,b,c
9	Borneol L	1136	0.82	0.61	1.49	a,b,c
10	Terpinene-4-ol	1141	0.46	0.16	-	a,b,c
11	Endoborneol	1248	-	-	0.08	a,b,c
12	Bornylacetate	1291	-	-	0.21	a,b,c
13	Bicycloelemene	1383	0.34	0.31	0.41	a,b,c
14	*α*-copaene	1421	0.88	0.47	0.08	a,b,c
15	*β*-elemene	1434	0.68	0.53	-	a,b,c
16	*α*-gurjunene	1442	1.69	1.33	0.54	a,b,c
17	Aristolene	1450	-	-	0.92	a,b,c
18	*β*-caryophyllene	1455	5.70	3.85	4.95	a,b,c
19	*β*-copaene	1458	-	-	0.30	a,b,c
20	*β*-gurjunene	1460	-	-	0.10	a,b,c
21	*β*-calarene	1461	-	-	0.40	a,b,c
22	*Trans*-*α*-bergamotene	1461	0.83	0.69	-	a,b,c
23	Aromadendrene	1463	0.36	-	2.16	a,b,c
24	Eremophilene	1465	-	-	0.31	a,b,c
25	6-*epi*-*β*-cubebene	1468	-	-	0.13	
26	*α*-humulene	1469	0.59	0.24	1.20	a,b,c
27	*Allo* aromadendrene	1471	0.30	0.42	1.88	a,b,c
28	γ-gurjunene	1472	-	-	2.40	a,b,c
29	*γ*-muurolene	1477	-	-	0.29	
30	*Cis*-*β*-guaiene	1478	-	-	0.18	a,b,c
31	*α*-amorphene	1481	0.61	0.16	-	a,b,c
32	Zingiberene	1483	1.24	0.45	0.67	a,b,c
33	Viridiflorene	1485	-	1.83	1.03	a,b,c
34	*α*-selinene	1484	-	-	0.14	
35	Bicyclogermacrene	1486	3.68	3.55	6.20	a,b,c

TABLE I - Chemical composition of *Tetradenia riparia* leaves, flower buds and stem essential oil

Peak	[a]Compounds	RI Calc	Relative area (%)			Methods of Identification
			Leaves	Flower buds	Stem	
36	*α*-muurolene	1488	0.84	0.48	0.00	a,b,c
37	*Trans*-*α*-farnesene	1490	0.51	0.33	-	a,b,c
38	*Cis*-*α*-bisabolene	1492	1.15	0.67	-	a,b,c
39	*γ*-cadinene	1494	1.29	2.07	2.44	a,b,c
40	*δ*-cadinene	1495	4.60	2.61	2.62	a,b,c
41	*Trans*-cadina-1,4-diene	1496	0.84	1.41	0.48	a,b,c
42	Eudesma-4(14),7(11)-diene	1500	-	-	0.42	a,b,c
43	Aromadendrene oxide-(2)	1507	-	-	0.50	a,b,c
44	Calarene epoxide	1513	-	-	0.16	a,b,c
45	Epiglobulol	1523	-	-	0.22	a,b,c
46	Isolongifolene oxide	1527	-	-	0.54	a,b,c
47	Palustrol	1531	-	0.18	0.40	a,b,c
48	Spathulenol	1543	-	-	9.00	a,b,c
49	Caryophyllene oxide	1548	1.32	4.50	4.55	a,b,c
50	Globulol	1551	-	1.08	0.27	a,b,c
51	Viridiflorol	1553	0.25	0.26	0.78	a,b,c
52	Carotol	1559	-	-	0.24	a,b,c
53	Ledol	1563	0.54	0.53	1.24	a,b,c
54	4-*epi*-cubebol	1569	-	-	0.55	a,b,c
55	T-muurolol	1601	3.43	3.90	3.11	a,b,c
56	*α*-cadinol	1602	12.21	13.69	7.31	a,b,c
57	*α*-acorenol	1689	-	-	0.34	a,b,c
58	caryophylla-4(12),8(13)-dien-5.beta.-ol	1692	-	-	0.79	a,b,c
59	*β*-Eudesmol	1693	-	-	1.90	a,b,c
60	δ-cadinol	1700	-	-	2.82	a,b,c
61	*Allo* aromadendrene oxide-(2)	1721	-	-	2.06	a,b,c
62	Cedrenol	1724	-	-	1.36	a,b,c
63	Caryophylla-3,8(13)-dien-5.beta.-ol	1732	-	-	0.53	a,b,c
64	1,6-germacradien-5-ol	1739	2.93	-	-	a,b,c

TABLE I - Chemical composition of *Tetradenia riparia* leaves, flower buds and stem essential oil

Peak	[a]Compounds	RI Calc	Relative area (%)			Methods of Identification
			Leaves	Flower buds	Stem	
65	Elema-1,3-dien-6α-ol	1745	-	-	4.88	a,b,c
66	γ-Costol	1756	-	-	3.93	a,b,c
67	Valerenol	1759	-	-	1.97	a,b,c
68	α-muurolol	1795	3.69	1.73	-	a,b,c
69	T-cadinol	1805	1.60	2.05	-	a,b,c
70	14-hidroxy-9-epi-caryophyllene	1897	8.56	15.38	4.29	a,b,c
71	Abieta-8,11,13-triene	2064	-	-	0.14	a,b,c
72	Abietadiene	2101	7.29	7.45	7.47	a,b,c
73	Calyculone	2192	0.28	0.22	0.20	a,b,c
74	9β,13β-epoxy-7-abietene	2231	1.43	2.90	-	d*
	Manoyl oxide	2290	0.31	0.45	-	
75	(1E,3Z,11E)-Cembra-1,3,11-trien-6-one	2361	-	0.60	-	a,b,c
76	6-7-Dehidro-royleanone	2392	5.80	9.61	0.04	d*
77	Anthracene,1,4-dimethoxy-9-phenyl	2444	-	0.99	-	a,b,c
	Total identified		**97.90**	**96.94**	**98.65**	
	Monoterpenes Hydrocarbon		6.09	1.33	4.48	
	Oxygenated Monoterpenes		16.04	8.69	2.12	
	Sesquiterpenes Hydrocarbon		26.13	21.40	30.25	
	Oxygenated Sesquiterpenes		34.53	43.30	53.74	
	Diterpenes Hydrocarbon		7.29	7.45	7.61	
	Oxygenated Diterpenes		7.82	13.78	0.24	
	Other compounds		-	0.99	0.21	

[a]Compound listed in order of elution from HP-5 column; [b]IR: Retention index calculated using n-alkane C9 – C30 in HP-5 column; [c]IR: Identification based on comparison of mass spectra using NIST 11.0 library; Relative area (%): percentage of the area occupied by the compounds in the chromatogram; t= trace; n.i = not identified; (-): absent. d *: identification by nuclear magnetic resonance (NMR) (Gazim et al., 2014).

The projection of the chemical classes (Figure 1) indicated the sesquiterpene hydrocarbon (26.13%; 30.25%) in greater quantity in the leaves and stem, respectively, and the oxygenated sesquiterpenes (43.30%; 53.74 %) in the flower buds and stem, respectively.

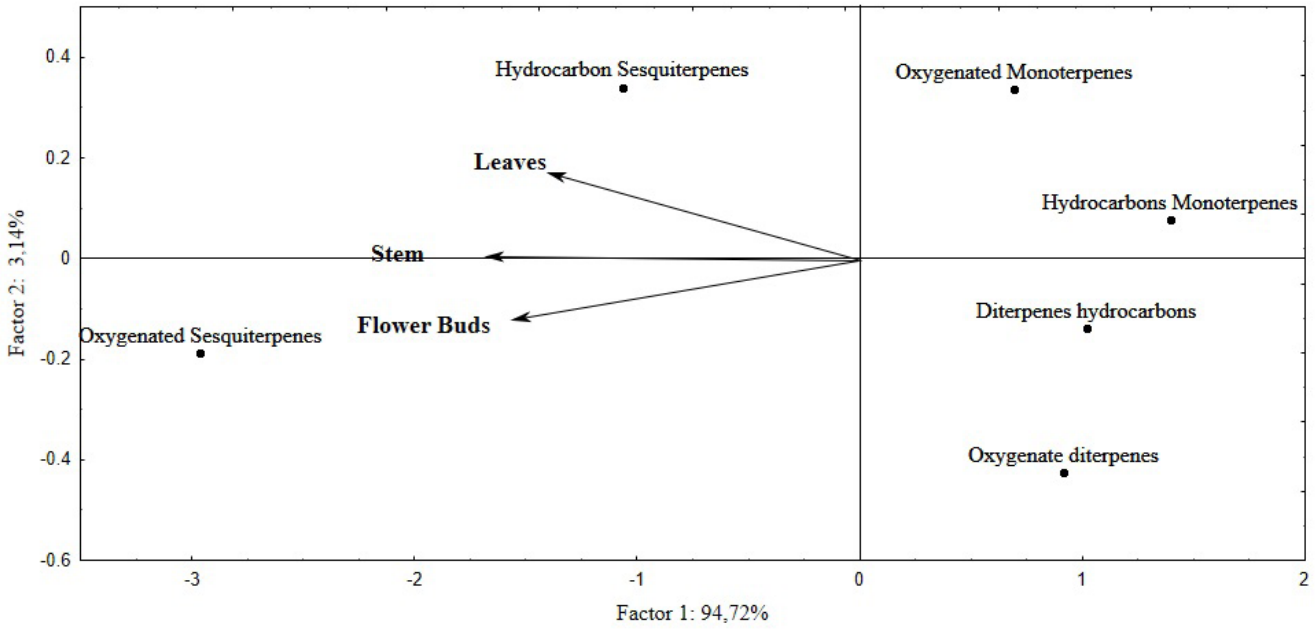

FIGURE 1 - Biplot of principal component analysis scores and loadings for the gas chromatography and mass spectrometry representing the projection of chemical classes of *Tetradenia riparia* leaves, flower buds and, stem essential oil.

The projection of chemical compounds (Figure 2) showed the presence of fenchone (11.57%) and 6-7-dehydro-roileanone (5.80%) in the leaves; 14-hydroxy-9-epi-caryophyllene (15.38%) and α-cadinol (13.69%) in the flower buds; spathulenol (9.00%) and bicyclogermacrene (6.20%) in the stem.

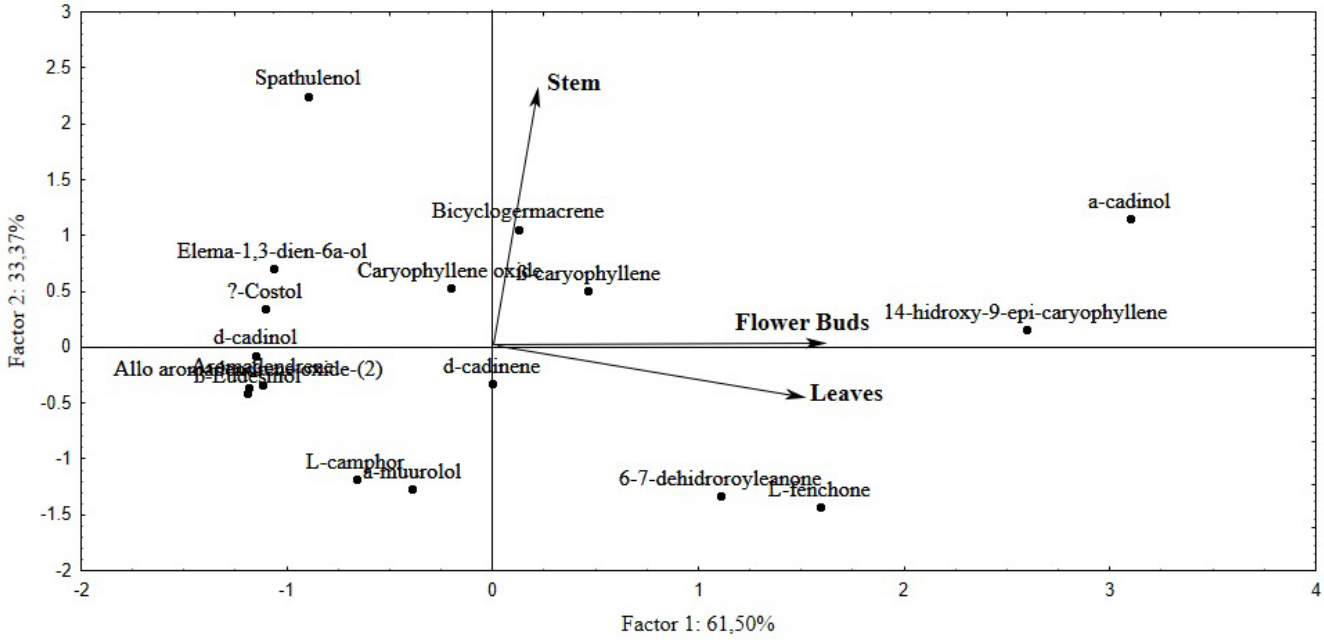

FIGURE 2 - Biplot of principal component analysis scores and loadings for the gas chromatography and mass spectrometry representing the projection of *Tetradenia riparia* leaves, flower buds, and stem essential oil.

T. riparia essential oils were evaluated against the larvae of *A. aegypti*, determining the lethal concentrations (LCs) necessary to eliminate 50.0% (LC_{50}) and 99% (LC_{99}); these results are listed in Table II.

The inhibition assay of acetylcholinesterase indicated that the EO of the leaves inhibited the enzyme up to 780 µg/mL, while the EOs of the flower buds and stem showed an effect up to 1560 µg/mL concentration.

TABLE II - Larvicidal activity by Larval Immersion Test (LIT) (%) and Lethal concentration LC_{50} and LC_{99} (µg/mL) and confidence interval (CI $\alpha = 0.05$) of *Tetradenia riparia* leaves, flower buds and stem essential oils that killed the larvae of *Aedes aegypti* by Probit analysis

Concentration (µg/mL)	Larvicidal activity (%)			
	Leaves (CI)	Flower Buds (CI)	Stem (CI)	Positive Control (CI)
12500	$100.0 \pm 0.0^{a,A}$ (99.6 ; 100.4)	$100.0 \pm 0.0^{a,A}$ (97.6 ; 102.4)	$100.0 \pm 0.0^{a,A}$ (99.5 ; 100.5)	$100.0 \pm 0.0^{a,A}$ (96.8 ; 103.2)
6250	$95.46 \pm 0.41^{b,B}$ (95.11 ; 95.81)	$99.51 \pm 0.31^{a,A}$ (97.11 ; 101.90)	$100.0 \pm 0.0^{a,A}$ (99.5 ; 100.5)	$100.0 \pm 0.0^{a,A}$ (96.8 ; 103.2)
3120	$79.03 \pm 0.58^{c,D}$ (78.68 ; 79.38)	$83.33 \pm 2.99^{b,C}$ (80.93 ; 85.72)	$95.90 \pm 0.38^{b,B}$ (95.41 ; 96.38)	$100.0 \pm 0.0^{a,A}$ (96.8 ; 103.2)
1560	$46.77 \pm 0.49^{d,D}$ (46.42 ; 47.12)	$64.49 \pm 3.27^{c,C}$ (62.10 ; 66.89)	$88.86 \pm 0.19^{c,B}$ (88.37 ; 89.34)	$100.0 \pm 0.0^{a,A}$ (96.8 ; 103.2)
780	$34.93 \pm 0.41^{e,D}$ (34.57 ; 35.28)	$53.23 \pm 2.42^{d,C}$ (50.83 ; 55.62)	$60.80 \pm 0.24^{d,B}$ (60.31 ; 61.28)	$95.00 \pm 5.00^{a,A}$ (91.80 ; 98.20)
390	$10.69 \pm 0.34^{f,D}$ (10.34 ; 11.05)	$36.03 \pm 3.08^{e,C}$ (33.64 ; 38.43)	$44.26 \pm 0.51^{e,B}$ (43.77 ; 44.74)	$75.00 \pm 5.00^{b,A}$ (71.80 ; 78.20)
195	$9.97 \pm 0.22^{f,C}$ (9.62 ; 10.32)	$26.06 \pm 4.69^{f,B}$ (23.67 ; 28.46)	$30.00 \pm 0.00^{f,B}$ (29.52 ; 30.48)	$57.67 \pm 2.08^{c,A}$ (54.46 ; 60.87)
97.5	$7.20 \pm 0.27^{g,D}$ (6.85 ; 7.55)	$15.22 \pm 0.25^{g,C}$ (12.82 ; 17.61)	$27.116 \pm 0.143^{g,B}$ (26.63 ; 27.60)	$52.33 \pm 2.52^{c,A}$ (49.13 ; 55.54)
48.75	$5.79 \pm 0.30^{h,D}$ (5.44 ; 6.15)	$10.23 \pm 0.26^{gh,C}$ (7.84 ; 12.63)	$23.16 \pm 0.29^{h,B}$ (22.68 ; 23.65)	$42.33 \pm 2.08^{d,A}$ (39.13 ; 45.54)
24.37	$1.49 \pm 0.005^{i,D}$ (1.14 ; 1.84)	$4.97 \pm 0.45^{h,C}$ (2.57 ; 7.36)	$20.44 \pm 0.58^{i,B}$ (19.95 ; 20.92)	$32.33 \pm 2.52^{e,A}$ (29.13 ; 35.54)
12.18	$0.40 \pm 0.07^{j,C}$ (0.048 ; 0.75)	$0 \pm 0^{i,C}$	$19.06 \pm 0.86^{j,B}$ (18.57 ; 19.54)	$24.00 \pm 3.61^{f,A}$ (20.80 ; 27.20)
6.09	$0 \pm 0^{j,B}$	$0 \pm 0^{i,B}$	$13.00 \pm 0.60^{k,A}$ (12.51 ; 13.48)	$13.67 \pm 3.21^{g,A}$ (10.46 ; 16.87)
3.04	$0 \pm 0^{j,B}$	$0 \pm 0^{i,B}$	$6.33 \pm 0.55^{l,A}$ (5.84 ; 6.81)	$6.00 \pm 2.65^{gh,A}$ (2.80 ; 9.20)
1.52	$0 \pm 0^{j,A}$	$0 \pm 0^{i,A}$	$0 \pm 0^{m,A}$	$0.667 \pm 0.577^{h,A}$ (0)
CN	$0 \pm 0^{j,A}$	$0 \pm 0^{i,A}$	$0 \pm 0^{m,A}$	$0 \pm 0^{i,A}$

TABLE II - Larvicidal activity by Larval Immersion Test (LIT) (%) and Lethal concentration LC_{50} and LC_{99} (µg/mL) and confidence interval (CI $\alpha = 0.05$) of *Tetradenia riparia* leaves, flower buds and stem essential oils that killed the larvae of *Aedes aegypti* by Probit analysis

Concentration (µg/mL)	Larvicidal activity (%)			
	Leaves (CI)	Flower Buds (CI)	Stem (CI)	Positive Control (CI)
	Lethal concentration (LC) (µg/mL)			
LC_{50}	1595^{C} (1444 – 1745)	675^{B} (636 –714)	665^{B} (645 - 684)	398^{A} (348- 448)
LC_{99}	11927^{D} (11767 -12073)	7120^{C} (6798 – 7442)	6289^{B} (6202 – 6376)	1140^{A} (1060 – 1200)

Values presented with average ± standard deviation. Averages followed by same letters in the same column and lines do not statistically differ by Tukey test with significance level of 5%. Different lowercase letters in column and uppercase letters in lines indicate that there were significant differences between treatments. CI: confidence interval. CP: Positive control, Temephós®-based commercial solution of organophosphate; CN: Negative control (2% aqueous polysorbate (80) solution). LC_{50}: concentration of oil that killed 50% of *Aedes aegypti* larvae; LC_{99}: concentration of oil that killed 99%;

DISCUSSION

The results of the action of the EOs (leaves, flower buds, and stem) on the larvae of *A. aegypti* indicated a significant difference in larval mortality between the essential oils evaluated. The EOs of the flowers bud and stem showed weak larvicidal activity with LC_{50} 675 and LC_{50} 665 µg/mL, respectively, and the oil from the leaves proved to be inefficient (LC_{50} 1590 µg/mL) (Table II).

For determining the larvicidal activity, the classification of these results is based on LC_{50} parameters established by Komalamisra *et al*. (2005), in which the compounds with $LC_{50} < 50$ µg/mL were considered highly active; compounds with LC_{50} between 50 to 100 µg/mL were considered moderately active; compounds with LC_{50} between 100 µg/mL to 750 µg/mL were considered effective; and compounds with $LC_{50} > 750$ µg/mL were considered inactive. These parameters are in accordance with Cheng *et al*. (2003), who standardized compounds with $LC_{50} < 50$ µg/mL as highly active and those with $LC_{50} < 100$ µg/mL as active. Kiran *et al*. (2006) considered as active compounds whose larvicidal effect was $LC_{50} < 100$ µg/mL. When comparing the results found in our experiment (Table II) with the parameters established in the literature, we can consider that the EOs of the stem and flower buds showed weak activity, and the leaves were inactive against the *A. aegypti* larvae.

This is the first study to examine the effect of *T. riparia* flowers bud, and stem EO on *A. aegypti* larvae. However, Fernandez *et al*. (2014) tested the activity of the EO of *T. riparia* leaves on *A. aegypti* larvae as a seasonal variation function over a year (2011 and 2012), finding larvicidal efficiency of the EO obtained in the autumn period (LC_{50} 79 µg/mL) and inactivity of the EO obtained in the winter period (LC_{50} 2620 µg/mL). The EOs used in our experiment were obtained from leaves, flower buds, and stem collected in the winter, justifying the inefficiency of the EO of the leaves (LC_{50} 1590 µg/mL) and weak activity of the stem (LC_{50} 665 µg/mL) and flower buds (LC_{50} 675 µg/mL) (Table II). The ideal would be to test the oils collected in other periods, as did Fernandez *et al*. (2014). They tested the oil in different climatic seasons, finding a high activity against *A. aegypti* larvae (LC_{50} 79 µg/mL) in the autumn. However, this species blooms only in winter, with no flower buds in the autumn season, with only leaves and stems.

The results found in the chemical composition and the principal component analysis (Table I and Figure 1) showed that oxygenated sesquiterpenes were predominant in the essential oils of the leaves (34.53%), flower buds (43.30%), and stem (53.74%). These results are in accordance with Gazim *et al*. (2010) and Fernandez *et al*. (2014), who also found the oxygenated sesquiterpenes (64.33%) and (40.49%) in the EOs of *T. riparia* leaves, respectively. Regarding the significant compounds, in our study, we found the oxygenated sesquiterpene 14-hydroxy-9-epi-caryophyllene in flower buds (15.38%) and leaves (8.56%); α-cadinol in flower buds (13.69%), leaves (12.21%), and stem (7.31%) and spathulenol (9.00%) only in the stem (Table I and Figure 2).

According to Paluch *et al*. (2009), one of the important functional roles of the various sesquiterpenes in plants is to protect against pests. Thus, the Japanese patent application no. 2000026210A includes spathulenol as an active compound and ingredient of an insecticide formula against *A. Aegypti* and *Culex pipiens pallens* L. (Diptera: Culicidae) (Dias, Moraes, 2013), which is in accordance with the present study, since spathulenol was one of the major compounds in the EO of the stem.

Simas *et al*. (2004) concluded in their study that oxygenated sesquiterpenes were more efficient on *A. aegypti* larvae when compared to monoterpenes and phenylpropanoids. These authors concluded that oxygenated sesquiterpene *trans*-nerolidol showed larvicidal potential (LC_{50} 13 μg/mL) when compared to monoterpene geraniol (LC_{50} 82 μg/mL) and phenylpropanoid safrole (LC_{50} 49 μg/mL).

Another compound class found in the EOs of *T. riparia* were hydrocarbons sesquiterpenes with 26.13% in the leaves, 21.40% in the flower buds, and 30.25% in the stem, exhibiting as major compounds β-caryophyllene and bicyclogermacrene in the three EOs (Table I; Figures 1 and 2). Doria *et al*. (2010) investigated the effect of the EO of *Croton pulegiodorus* Baill (Euphorbiaceae) on *A. aegypti* larvae, finding LC_{50} of 49 μg/mL; chemical analysis revealed the presence of β-caryophyllene (20.96%) and bicyclogermacrene (16.89%). Lima *et al*. (2011) and Kiran, Devi (2007) pointed out in their studies that there is a direct relationship between the larvicidal effect and high concentrations of hydrocarbons sesquiterpenes.

Monoterpenes were also highlighted in the EOs analyzed in our study. Tripathi, Mishra (2016) carried out a review of monoterpenes' insecticidal potential, stating that some monoterpenoids appear to be important in the development of domestic pesticides. A monoterpene that may have contributed to a synergistic effect was limonene; according to Santos *et al*. (2011) and Cheng *et al*. (2009), limonene has been widely used to control mosquitoes of the genus *Aedes*. In addition, limonene is a low toxicity compound that has been recorded as an active ingredient in larvicide products such as insecticides and insect repellents for use in humans and in the control of fleas and ticks for pets (Epa, 1994).

The diterpenes identified in the EOs of *T. riparia* leaves, flower buds, and stem (Table I; Figures 1 and 2) may have contributed to the larvicidal potential found. This class has stood out due to its diverse biological effects, including anti-tumor (9β, 13β-epoxy-7-abietene) and antioxidant (6,7-dehydroroyleanone); these two diterpenes were found in the EO of *T. riparia* leaves and they were identified by Gazim *et al*. (2014).

Recent studies conducted by Islam, Mubarak (2019) demonstrated the effect of diterpenes and their derivatives as promising agents against the dengue virus and its vectors. These authors suggest that several diterpenes and/or their derivatives act against the dengue virus and its two potential vectors, namely, *Aedes aegypti* and *A. albopictus*.

The larvicidal potential can be explained by the effect of terpenes, which are substances that increase the transmembrane absorption of both lipophilic and hydrophilic drugs. The lipophilic effect of terpenes plays a fundamental role in the modulation of larvicidal activities. The association between lipophilic compounds and protein deactivation/enzyme inhibition may explain the activity (Ryan, Byrne, 1988).

Another point concerns the influence of terpenoid volatility and its influence on the larvicidal response. In this sense, Sharma *et al*. (2019) reported that sesquiterpenes have lower volatility than monoterpenes; thus, they remain in contact with the larvae longer, which intensifies the larvicidal potential. The present work evaluated the probable mechanism of action of EOs, measuring their inhibitory potential upon the enzyme acetylcholinesterase. The results indicated that the EO of the leaves inhibited the enzyme up

to 780 µg/mL, while the EOs of the flower buds and stem inhibited the enzyme up to 1560 µg/mL. The results of the inhibition of acetylcholinesterase *in vitro* were superior to those found in larvae *in vivo.* This difference is justified by the absence of physiological conditions that interfere with the insect's biochemical reactions, since the bioautography protocol is performed in a controlled environment with all pre-established conditions, without the interference of cell wall permeability, the size and the solubility of molecules, in hydrophilic and lipophilic media (Brain *et al*., 2007).

Larvicides used in vector control mainly belong to groups of growth regulating larvicides, such as inhibitors of chitin synthesis and juvenile hormone analogues (Pyriproxyfen) and organophosphates (Temefós, Malathion 44%) (OMS, 2019). The interest in establishing a mechanism of action for the EOs of *T. riparia* is related to the mechanism of action of larvicides for the control of pests and insects, based on the need for larvicides that are less harmful to the environment and that do not have negative health impacts when compared to synthetic larvicides (Ibrahim *et al*., 2001). Considering that the larvicides currently used are synthetic insecticides, the larvicidal activity of the essential oil of the three plant parts of *T. riparia* opens new perspectives for the search of natural compounds with the potential to control *A. aegypti* larvae. Given the results found, further studies should be conducted to develop a bioinsecticide.

CONCLUSIONS

Monoterpenes, sesquiterpenes, and diterpenes were identified in the essential oil of the leaves, flower buds, and stem of *T. riparia* collected in winter season, with sesquiterpenes as the major class. When tested on *A. aegypti* larvae, the stem and flower buds essential oil showed weak action with LC_{50} 675 and 665 µg/mL, respectively; the leaves were inactive against the *A. aegypti* larvae with LC_{50} 1560 µg/mL.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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